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RESEARCH ARTICLE

Capsaicin from *Capsicum annuum* var. Cayenne: Isolation, GC-MS Analysis, Anticancer Activity on MG63 Osteosarcoma Cells, and *In Silico* Interaction with 6T2W

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Abstract

Background: *Capsicum annuum* (cayenne pepper), a member of the *Solanaceae* family, is known for its bioactive compounds, particularly capsaicin, which have demonstrated anticancer potential. This study aimed to explore the cytotoxic effects of cayenne pepper's bioactive components on osteosarcoma cells and investigate the molecular mechanisms underlying their activity using advanced computational tools. **Aim:** This study's main goal was to identify and isolate bioactive substances from *C. annuum*, evaluate their cytotoxic effects on MG-63 osteosarcoma cells, and explore the binding interactions between capsaicin and the 6T2W protein using molecular docking. **Methods:** Dried cayenne pepper fruit was powdered and subjected to ethanol extraction to isolate its bioactive components. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified seven compounds, including capsaicin, dihydrocapsaicin, linolelaidic acid, and hexadecanoic acid. The cytotoxicity of Capsaicin was evaluated using the MTT assay on MG-63 osteosarcoma cells. Molecular docking studies, conducted using ChemDraw Ultra 8.0, AutoDock Vina, and Pyrx Virtual Screening Tools, examined capsaicin's interaction with the active site of the 6T2W protein. **Results:** The IC_{50} values determined from the MTT assay showed capsaicin to be the most potent compound with an IC_{50} of 50 µg/ml. Molecular docking revealed strong interactions between capsaicin and the 6T2W protein, suggesting a potential mechanism for its anticancer activity. **Conclusion:** Capsaicin and the mother extract from cayenne pepper demonstrated the highest cytotoxicity against MG-63 osteosarcoma cells, positioning them as potential candidates for osteosarcoma therapy. The molecular docking studies offer insights into the binding mechanisms of capsaicin with the 6T2W protein, paving the way for further investigation into its therapeutic applications. **Major Findings:** Capsaicin was successfully isolated from *C. annuum* var. Cayenne and confirmed through GC-MS analysis. It exhibited significant cytotoxic activity against MG63 osteosarcoma cells and showed strong binding affinity to the 6T2W protein target in molecular docking studies.

Keywords: Anticancer Activity, Capsaicin, *Capsicum annuum var.* Cayenne, *In Silico* Interaction, GC-MS Analysis, Osteosarcoma (MG63)

1. Introduction

Cancer is the second leading cause of mortality for both men and women, accounting for over 9.8 million deaths globally[1-3]. With an annual incidence of 3.4 occurrences per million persons worldwide, Osteosarcoma (OS) is a rather uncommon bone tumour. Classic OS had extremely poor 5-year survival rates of 56.31% during

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most of the 20th century[4]. The largest incidence of this cancer type occurs between the ages of 10 and 30, making up around 2.4% of all paediatric malignancies. Originating from mesenchymal cells, it frequently affects long bones including the humerus, tibia, and femur. OS has a little male predominance and is more prevalent in Asian/Pacific Islanders, Hispanics, and African Americans than in the White population. Although the exact cause of osteosarcoma is still unknown, some epidemiological risk factors have been linked to a higher chance of developing the condition. The most common primary malignant bone pathology, osteosarcoma, is characterised by its aggressiveness. Other risk factors like radiation exposure, fibrous dysplasia, and Paget's disease of the bone are also linked to an increased risk, especially in older populations[5,6]. It's noteworthy to observe that taller persons are more likely to have osteosarcoma than the overall population[7]. Although osteosarcoma has numerous subtypes, most of them are high-grade and exhibit aggressive biological activity[8]. Despite improvements in OS treatment, results are still not optimal.Despite advances in surgical techniques and chemotherapy, the prognosis for patients with metastatic or recurrent osteosarcoma remains poor, with five-year survival rates below 30%. Challenges in treatment include chemoresistance, significant side effects of conventional therapies, and the lack of targeted treatment options. These limitations underscore the urgent need for novel therapeutic approaches, including those derived from natural products with anticancer potential[9]. Plant-based bioactive compounds have emerged as a valuable resource in the development of new therapeutics due to their diverse chemical structures and biological activities. Their ability to modulate multiple cellular pathways, target specific proteins, and exhibit lower toxicity compared to synthetic drugs makes them promising candidates for cancer therapy. Leveraging such compounds provides an opportunity to discover novel, cost-effective, and sustainable treatments for various diseases, including cancers like osteosarcoma[10]. Numerous studies have demonstrated that a wide range of plant species contain a diverse array of bioactive chemicals with a range of biological functions[11-14]. According to recent studies[15-17], the pharmaceutical industry has taken into consideration natural compounds derived from medicinal plants because of their anti-inflammatory, anti-tumour, and anti-microbial qualities. They are now a valuable source of components for the development of novel drugs due to their biological activity. As one of the earliest cultivated plants, peppers have been utilised by humans from prehistoric times, according to archaeological remains[18]. Previous studies have assessed the extracts of *C. annuum* for toxicity, carcinogenicity, and anti-tumour properties[19-21]. Based on their research, Red chilli (*C. annuum*) was found to be generally safe at the levels under investigation[20]. Cayenne pepper is a type of *C. annuum*, family *Solanaceae*. Research has shown that peppers are rich in bioactive substances with antioxidant properties, including carotenoids, flavonoids, provitamin A, vitamin C, and vitamin E. These chemicals help to reduce harmful oxidative stress. These substances may also be able to protect against a number of illnesses linked to the oxidation of free radicals, including cancer, neurological problems, and cardiovascular disease[22]. *C. annuum var a* plant with substantial medical value, cayenne is recognised for its abundance of bioactive substances, including phenolics and capsaicin, which have strong anti-inflammatory, anti-cancer, and antioxidant effects. The main active ingredient, capsaicin, has shown promise in causing apoptosis and halting the spread of cancer, while phenolic compounds contribute to reducing oxidative stress and enhancing cellular defence mechanisms. These attributes make *C. annuum* var. Cayenne is a promising candidate for exploring natural therapeutic solutions, particularly for challenging diseases like osteosarcoma. The objectives of this study are to isolate capsaicin from *C. annuum* var. Cayenne, conduct molecular docking studies on cancer-related targets (6T2W28), and profile the bioactive compounds present using GC-MS. Additionally, the study aims to evaluate the anticancer effects of capsaicin isolated from the ethanol extract of dried Cayenne Pepper fruit on MG63 osteosarcoma cells, assessing its potential to inhibit cell proliferation and induce apoptosis.

2. Materials and Methods

2.1 Collection of Fruit Material

The dried cayenne pepper fruit (Figure 1) was collected from authorized source and verified by Dr. P Elankani,



Figure 1. Dried cayenne pepper fruit.

the research officer-Siddha, Sci-1V/incharge, at the Siddha Central Research Institute in Chennai, Ministry of AYUSH, Government of India, and Dr. KN Sunil Kumar, the research officer/Sci-11, HOD Department of Pharmacognosy.

2.2 Preparation of Mother Extract

The fruit was collected and left to dry in the shade for 15 days at room temperature, then it was ground into a coarse powder with an electrical grinder, and the medication was run through filter No. 2213 to ensure a consistent particle size. 250 ml of 95% ethanol was used to extract 50 g of cayenne pepper fruit powder in an Erlenmeyer flask over the course of 24 hours[23]. Subsequently, A Whatmann filter paper No. 2 was used to filter the mixture and concentrated using a rotary evaporator. It was then kept in a refrigerator at 4°C until it was required for additional analysis.

2.3 Isolation of Chemical Constituents

Capsaicin can be isolated from cayenne pepper using Thin Layer Chromatography (TLC). Transfer the mother extract onto a TLC plate made of silica gel. Put the plate in a developing chamber with a mobile phase inside it, such as a hexane-ethyl acetate mixture. Allow the solvent to rise, carrying the compounds along the plate. Once developed, visualise the spots under UV light or iodine vapour. The spot corresponding to capsaicin can be identified by its R_f value compared to a known standard. In the TLC analysis of silica on plastic (eluting with dichloromethane/MeOH, 10:1), seven different spots were seen, suggesting that the extract combination contained at least seven different compounds. The extract sample, extract mixture, and collected eluents[24] were sent for GC-MS analysis after column chromatography (silica gel, eluting with dichloromethane/MeOH, 10:1).

2.4 GC-MS Profiling

The fruit extract containing cayenne pepper was subjected to GC-MS analysis using Thermo MS DSQ II and Thermo GC-Trace Ultra Version: 5.0. The instrument comes with a non-polar column, DB 35-MS Capillary Standard, which measures 30 mm × 0.25 mm ID × 0.25 µm film. The carrier gas, helium, moves at the slow rate of 1.0 millilitres per minute. The injector was operated at 250°C for 15 min because it was the temperature at which the oven was configured to operate. Then, during the next three minutes, the temperature increased gradually to 280 °C. For component identification, Willey and NIST libraries were utilised, and their retention indices were contrasted. The results were consistent once the components of the GC-MS instrument were identified and compared with those in the computer library (Willey and NIST)[25].

2.5 In Silico Analysis

2.5.1 Software Used in Docking Study

ChemDraw Ultra 8.0, AutoDock vina, and the Molecular Graphics Laboratory (MGL) tools were downloaded from Pyrx Virtual Screening Tools. Swiss ADME was used to translate proteins into PDB format, whereas Chem 3d Pro 8.0 was used to translate ligand Mol files into PDB format[26].

2.5.2 Protein Required

The binding energies of the chosen compounds, Capsaicin on the crystal structure, were obtained in 1971 for PDB ID: 6T2W[27] (Protein bicaudal D - Drosophila melanogaster) from the Protein Data Bank at Brookhaven National Laboratory.

2.5.3 Introduction

ADT is a programme of automated docking tools that is intended to forecast the manner in which small molecules will attach to a target protein with a known three-dimensional structure. AutoDock vina

was used to ascertain the binding modes of those recently produced compounds in order to calculate their binding energies in the active sites. With the Discovery Studio visualizer, the ligand's location within the enzyme binding site may also be seen[28]. It might be helpful to understand the binding nature as well as to develop possible medication candidates. Using molecular docking analysis, the developed compounds were able to predict the anticancer action of the bone on the crystal structure of PDB ID: 6T2W.

2.5.4 Methodology

Computer-aided drug design is one technique that is crucial for understanding the relationship between structure and activity, binding energy, ligand-protein interaction, binding affinity, etc. Auto dock was heavily used on this programme to evaluate the binding studies of our compound on the intended enzyme[29].

2.5.5 Preparation of Protein

The water molecules were taken out of the corresponding proteins prior to the molecular docking process beginning. The Swiss PDB viewer was used to further refine the chosen enzymes from the Protein Data Bank. The 6T2W structure represents the Bicaudal D (BicD) protein structure of Drosophila melanogaster in the F684I mutant.Due to the coiled-coil registry change that this mutant causes, dynein motility is activated without the need for cargo. This structural alteration facilitates comprehension of the mechanism by which BicD activates dynein, a motor protein implicated in intracellular transport[30].

2.5.6 Preparation of Ligand

Chem draw (Cambridge, MA, USA) was used to sketch the ligand structures. Hydrogen atoms were added and water molecules were subtracted from the protein file (pdb) in order to further optimise it. After completing all of these steps, the ligand and protein were ready for docking[31].

2.5.7 Receptor Grid Generation

A "prepared" structure,that is, an all-atom structure with the proper bond ordering and formal charges,is necessary for receptor grid creation. AutoDock searches for beneficial interactions between one or more ligand molecules and a receptor molecule, usually a protein. Multiple sets of fields are used to depict the receptor's shape and properties on a grid, allowing for increasingly precise ligand pose scoring. Using the tabs on the Receptor Grid Generation page, you can specify the receptor structure by removing any possible co-crystallised ligand. The position and size of the active site, which will be depicted by receptor grids, can also be ascertained. Additionally, AutoDock limitations can be set. A grid zone formed around the binding site of the receptor[32].

2.5.8 Docking Validation

Auto Dock Vina was used on the prepared crystal structures of the ligand andactive sites of several distinct enzymes, including the crystal structures of [PDB ID: 6T2W], to ascertain the binding energies. For the research, the docking grid box was roughly set above 90 using the Genetic Algorithm's (GA) default parameters. The number of runs and the other options in the search parameter were left at their default values. The output displayed the docking calculations' results in Word format. The ligand conformation that displayed the lowest binding energy (best posture) was chosen in order to determine the conformation of the ligand docking results. Using AutoDock techniques, the location and orientation of ligands within protein receptors, as well as their interactions with amino acids that bind to the ligand, were examined and depicted. Following the energy minimisation, the top 10 conformations for each compound were simulated during the docking procedure[33].

2.6 Cytotoxicity Study

2.6.1 Cell Line Used

In biomedical research, the MG-63 human osteosarcoma cell line, which NCCS Pune provides, is frequently employed. These cells, which are derived from the osteosarcoma of a 14-year-old Caucasian man, have a high rate of proliferation and osteoblast-like properties. They are widely employed in studies related to drug screening, cancer biology, and bone-related disorders[34].

2.6.2 Cell Line Maintenance

A culture medium fortified with Foetal Bovine Serum (FBS), such as Dulbecco's Modified Eagle Medium (DMEM), antibiotics, and additional growth factors (MG-63), is necessary for osteosarcoma cell lines. MG-63 cells are used in research on osteosarcoma, cancer metastasis, bone production and resorption, and the impact of different therapies. The cells were cultivated in T25 culture flasks using DMEM, RPMI, 10% FBS, and 1% antibiotics. The cells were kept in a humidified environment with 5% CO_2 at 37°C. Following confluency and trypsinisation, the cells were passaged[35].

2.6.3 Cell Viability (MTT) Assay

To test for cell survival, the osteosarcoma cell line was exposed to capsaicin, which was isolated from dried cayenne pepper fruit using the solvent ethanol, using the MTT method. The assay relies on metabolically active cells reducing soluble yellow tetrazolium salt to insoluble purple formazan crystals. The osteosarcoma cell line was plated in 96-well plates at a density of 5x103 cells/well. Following plating, the cells were starved for three hours at 37°C in serum-free media and rinsed twice with 100µl of the medium. Following a full day of starvation, the cells were exposed to different amounts of capsaicin (5-100µg/ml) and test chemicals (0.1-1000µg/mL).20µL of MTT solution (5mg/mL) was applied to each plate well after a 48-h incubation period, and the plates were thereafter maintained in an incubator. To dissolve the purple formazan crystals, 100µL of DMSO solvent was applied to each well after four hours. The plates were then scanned at 570 nm using a Synergy HT Microplate Reader (Bio-Tek Instruments, Winooski, VT) set to 690 nm as the reference wavelength. Sigma Plot 12.0 was utilised to calculate the IC_{50} values. The percentage of control cells cultured in serum-free media was used to determine the number of viable cells. The cells in the control media remained alive after no treatment at all[36]. Cell viability is calculated using the formula below:

% cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100

2.6.4 Statistical Analysis

All of the data were examined using a One-way ANOVA and a Student's t-test in SPSS; the result was the mean ± SD for the three sets of data. The statistical significance criterion was set at $p<0.0526$.

3. Results

Our research program has started with the goal of separating natural compounds from plant or marine sources and examining their cytotoxic effects to determine whether they have anti-tumour effects. We have selected the dried cayenne pepper fruit (*C. annuum*, *Solanaceae*) for our study line. The biological activities of this species have been the subject of numerous investigations by researchers, but to the best of our knowledge, the anti-tumour properties of the plant extracts have received less attention. We therefore decided to investigate the cytotoxicity of *C. annuum* on MG-63 Cells as well as its separated natural products in light of the research on extracts from the plant. We used a fruit powder with dried cayenne pepper for this (Figure 1).

3.1 Isolation of Chemical Constituents

In the TLC analysis of silica on plastic (eluting with dichloromethane/MeOH, 10:1), seven distinct spots were seen, suggesting that the extract combination contained a minimum of seven different compounds. After that, the extract sample was examined using column chromatography (silica gel) and eluted with a solution of 10:1 dichloromethane/meOH. Together with the gathered eluents, the extracted mixture was sent for GC-MS analysis.

3.2 GC-MS Approach

GC-MS analysis of seven substances with retention times ranging from 11.212 to 34.569 was detected, as Table 1 illustrates. Capsaicin (81.55%) had the highest peak (%), followed by Dihydrocapsaicin (3.29%), Linoelaidic acid (2.73%), Hexadecanoic acid (4.52%) methyl ester, Hexadecane (4.5%), Dodecane, 4,6-dimethyl (1.90%), and Decane (1.51%). Four phytoconstituents were found amongthe seven peaks that were identified by comparing them with the NIST library.

Table 1. GC-MS analysis of ethanolic extract spectral data of the dry fruit of cayenne pepper

RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area%
34.351	Capsaicin	$C_{18}H_{27}NO_3$	305	81.55
30.569	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	307	3.29
30.463	Linoelaidic acid	$C_{18}H_{32}O_2$	280	2.73
27.631	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	4.52
22.658	Hexadecane	$C_{16}H_{34}$	226	4.5
17.211	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$	198	1.90
11.212	Decane	$C_{10}H_{22}$	142	1.51
Total				100

In Gas Chromatography-Mass Spectrometry (GC-MS), high and low retention times provide information about the properties of the materials being studied.A longer time for the chemical to pass through the GC column is indicated by a high retention time. Higher retention durations are typically associated with chemicals that are bigger, more non-volatile, or exhibit stronger interactions with the column's stationary phase. Higher molecular weight, increased polarity (assuming the stationary phase is polar), or stronger interactions like hydrogen bonds or van der Waals forces could be the cause of this. Big molecules of organic compounds, chemicals with strong interactions with the column material or compounds with a high boiling point. A short retention period indicates a fast compound flow across the GC column. Lower retention time compounds are typically smaller, less strongly interacting with the stationary phase, or more volatile. These substances often have lower boiling temperatures and molecular weights. Small chemical molecules, substances with weak interactions with the column material or compounds with a low boiling point. To summarise, retention time is a crucial component of GC-MS that represents the physical and chemical characteristics of molecules, impacting their identification, quantification, and separation.

3.3 InSilico Analysis

3.3.1 Docking Analysis on 6T2W

The molecular docking studies were carried out for Capsaicin against [PDB ID: 6T2W].The F684I mutant of Drosophila melanogaster's Bicaudal D (BicD) protein structure is represented by the 6T2W structure. The binding energy between the compound Capsaicin and 6T2W was found to be -6.8 (kcal/mol). This mutant activates dynein motility without the need for cargo by causing a coiled-coil registry change. This structural change contributes to our understanding of how BicD stimulates the motor protein dynein, which is involved in intracellular transport. The results afforded an excellent binding score,as mentioned in Table 2.

According to the amino acid residue interaction, all compounds showed hydrogen bond interactions, and some of them also showed van der Waals attraction in different amino acid residues at the binding site. Universal compounds were shown to have better binding affinity and interaction because the amino acid binding site has more hydrogen bonds and van der Waals. The most active molecule exhibited hydrogen bond interactions over the enzyme, despite the fact that pi-alkyl and pi-sigma interactions were also investigated (Table 3).

One of the most widely used docking software tools, auto AutoDock Vina, was used to anticipate each ligand's binding energy against the intended macromolecule. This resulted in eight different binding poses. The ligand's binding affinity was then determined by taking the binding position with the highest binding energy that yielded an RMSD value of zero. The best binding energy in docking studies is between -6.57 and -11.11 kcal/mol, the average binding energy is between -3.84 and -7.41 kcal/mol, and the poor binding energy is between 0.19 and 4.98 kcal/mol. The compounds have good bioavailability, no carcinogenicity, and are AMES negative in addition to having good HIA (Human Intestinal Absorption) and B.B.B. (Blood Brain Barrier) properties. The good drug similarity properties are also not broken by the chosen ligands.

Table 2. Docking result and interacting sites of tested compounds on 6T2W

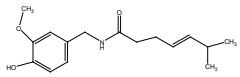
Compound code	Structure of the testedcompound	Binding score (kcal/mol) and PDB ID
CPN		6T2W -6.8 (kcal/mol)

Table 3. Docking result and various interactions of the tested compounds on the targeted proteins

Compound code		Vander Walls	H.bond	Pi-alkyl	Pi-sigma
CPN	6T2W	ASP A:625 ASN A:814 GLN A:547 ALA A:815 GLY A:591, 594,798 GLU A:626 VAL A:548 LEU A:799	PHE A:593 ALA A:592	ALA A:629 ARG A:816	ILE A:803

All compounds exhibited hydrogen bond interactions, and some of them further showed van der Waals attraction at various amino acid residues at the binding site, according to the amino acid residue interaction. It has been demonstrated that universal compounds have almost larger binding affinity and interaction because the amino acid binding site has more hydrogen bonds and van der Waals. Though pialkyl and pi-sigma interactions are studied, the most active molecule's interactions with the enzyme are dominated by hydrogen bond interactions. A good binding energy is present for the selected compounds (CPN) against (PDB ID: 6T2W) for anti-cancer activity. The binding energy between the compound Capsaicin and6T2W was found to be -6.8 kcal/mol (Figure 2). Nonetheless, the substances generated superior binding energy in relation to the intended macromolecule. This indicates that the capsaicin from dried fruit Cayenne Pepper's ethanolic extract has a high binding energy, which helps the substance outcompete competing molecules for the target molecule's binding. A compound with a higher negative binding energy is more stable. Cayenne pepper has therefore been shown to have a good action on cancer cells.

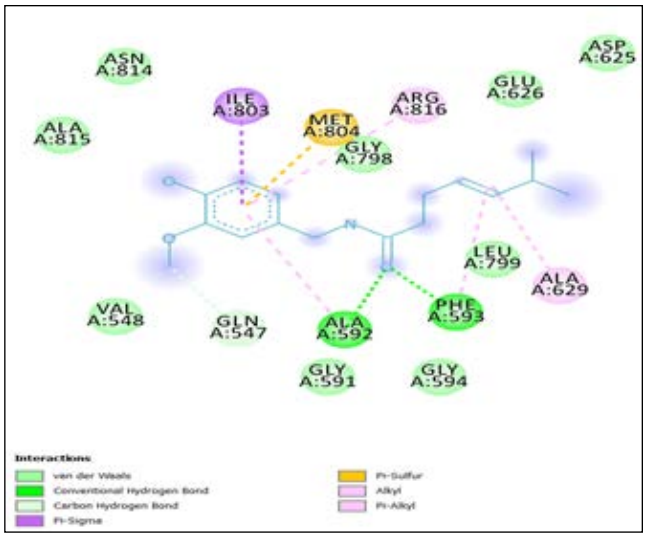


Figure 2. 2D stereoview of compound CPN on enzyme 6T2W.

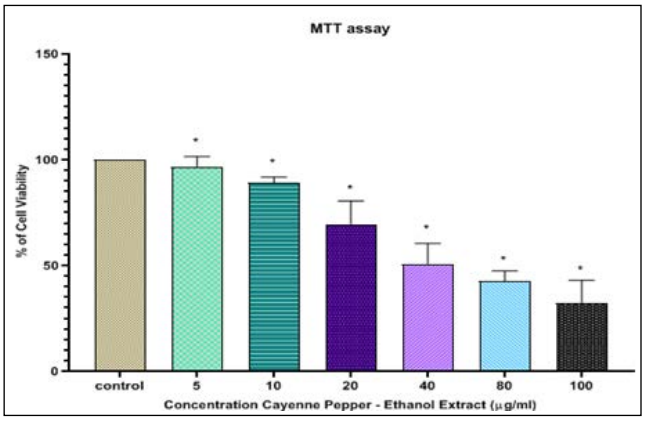


Figure 3. Capsaicin on the cell viability of human osteosarcoma cells (MG-63).

3.4 Cell Viability Assay – Osteosarcoma Cell Line (MG-63 cells) – Capsaicin

Using an MTT assay, the impact of Capsaicin on the viability of osteosarcoma cells was evaluated. For twenty-four hours, cells were exposed to different doses of capsaicin from the dried Cayenne pepper fruit extract (5 ug/ml, 10 ug/ml, 20 ug/ml, 40 ug/ml, 80 ug/ml, and 100 ug/ml) (Figure 3). After incubation, the IC_{50} value was obtained at a concentration of 47.127 ug/ml.

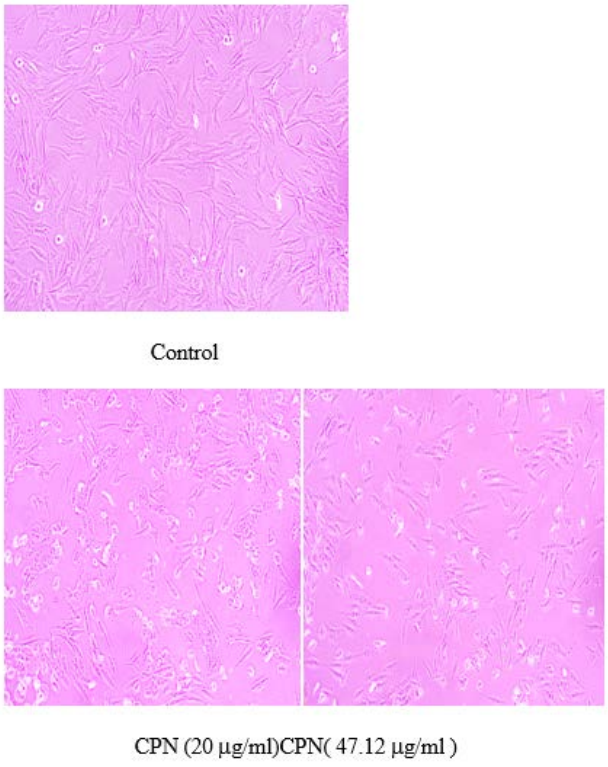


Figure 4. Effect of CPN on cell morphology of human osteosarcoma cells (MG-63) in the concentration of 20 µg/ml and 47.12 µg/ml.

3.4.1 Morphology Study

We identified the optimal doses from the capsaicin (IC_{50}: 47.127 µg/ml for osteosarcoma cell line and 20 and 47.12 µg/ml for MG-63 cell line) based on the MTT assay. (examining alterations in cell shape using a phase contrast microscope. For a full day, 2 × 105 cells were cultivated on six-well plates and subjected to capsaicin. The medium was taken out of the cells after the incubation time, and phosphate buffer saline (PBS pH 7.4) was used to clean them once more. A phase contrast microscope was used to look at the plates. 42.

3.4.2 Effect of Capsaicin on Cell Morphology of Human Osteosarcoma Cells (MG-63)

The MTT test was employed to evaluate the cellular viability subsequent to a 24-h treatment with CPN (5-100µg/ml). The means±SD (n=3) are displayed for the data. *p<0.05 in comparison to the blank control group. For a whole day, cells were treated with CPN (20 and 47.12µg/ml) under an inverted phase contrast microscope. After being treated with CPN, the cells shrank and showed signs of blebbing in the cytoplasmic membrane (Figure 4).

To sum up, research on osteosarcoma is essential to raising survival rates, creating safer and more effective treatments, and eventually raising the standard of living for those who are afflicted with this aggressive illness. The possible anti-cancer properties of cayenne pepper dried fruit are mainly ascribed to its active components, such as dihydrocapsaicin and capsaicin. According to this study, these substances may slow the spread of numerous cancer cell lines and the growth of cancer cells. They may also cause apoptosis, or programmed cell death. The protective effects of capsaicin and similar chemicals against the development and progression of cancer are attributed to their anti-inflammatory and antioxidant characteristics. Using an MTT assay, the impact of Capsaicin in ethanol extract from dried Cayenne pepper fruit on the viability of osteosarcoma cells was evaluated. For twenty-four hours, cells were exposed to different doses of CPN (5 ug/ml, 10 ug/ml, 20 ug/ml, 40 ug/ml, 80 ug/ml, and 100 ug/ml).A dose-dependent decrease in cell viability was observed with increasing concentration of CPN. The IC_{50} value of CPN was calculated approximately at 47.127 ug/ml. After 24 h, at the concentration of 47.127 ug/ml, 50% of the cells were killed. Under an inverted phase contrast microscope, cells were treated with CPN (20 and 47.12µg/ml) for the duration of the day. The number of cells decreased after receiving CPN, and they showed signs of cell shrinkage and cytoplasmic membrane blebbing. Future research should explore the efficacy and safety of Capsaicin from dried Cayenne pepper fruit across multiple osteosarcoma cell lines and patient-derived xenograft models. This study demonstrates that capsaicin in the extract exhibits strong anti-tumour effects on osteosarcoma cells, aligning with previous findings on its anti-cancer properties. Capsaicin's ability to inhibit cell growth and viability suggests its potential as an adjunct therapy for osteosarcoma. Further *in vivo* studies and clinical trials are needed to confirm these results and elucidate the underlying mechanisms.

4. Discussion

Capsaicin, the active compound in *C. annuum* var. Cayenne, shown promising anticancer potential[37] when

compared to other known agents such as curcumin, resveratrol, and doxorubicin. Like these agents, capsaicin induces cancer cell death through mechanisms such as apoptosis and cell cycle arrest. Existing literature demonstrates that capsaicin can inhibit cancer cell proliferation, induce apoptosis, and suppress tumour growth in various cancer models[38]. The mechanisms through which capsaicin exerts its effects include the activation of pro-apoptotic pathways, such as caspase activation, and the inhibition of anti-apoptotic proteins like Bcl-2[39]. Additionally, capsaicin induces cell cycle arrest at the G1 phase, disrupting the progression of cancer cells. These findings highlight capsaicin's potential as a therapeutic agent for cancer treatment. Molecular docking results reveal that capsaicin binds effectively to key cancer-related targets, such as 6T2W, with high binding affinity, suggesting its potential as a cancer-targeting molecule. The interaction of capsaicin with these targets, including hydrogen bonds and hydrophobic interactions, implies its ability to disrupt cancer cell signalling and promote apoptosis. The docking results align with experimental data showing capsaicin's anticancer effects, such as its induction of cell death and inhibition of cell proliferation. Further validation with literature supports the docking findings, demonstrating capsaicin's capacity to modulate cancer-related pathways, reinforcing its therapeutic potential in cancer treatment[40]. GC-MS profiling of *C. annuum* var. Cayenne extract revealed several bioactive compounds, including dihydrocapsaicin, linoelaidic acid, hexadecanoic acid methyl ester, hexadecane, dodecane, 4,6-dimethyl-, and decane. Dihydrocapsaicin, a close analogue of capsaicin, may enhance its anticancer effects by acting through similar mechanisms. Linoelaidic acid and hexadecanoic acid methyl ester possess anti-inflammatory and antioxidant properties, potentially supporting capsaicin's anticancer activity. Hydrocarbon compounds like hexadecane, dodecane, and decane might contribute to the extract's ability to modulate cell membrane integrity and increase the bioavailability of capsaicin. The interactions between these compounds could amplify capsaicin's anticancer effects, leading to a more robust inhibition of cancer cell proliferation and enhanced apoptosis. The findings from this study highlight the potential of capsaicin and other bioactive compounds from *C. annuum* var. Cayenne is a promising therapeutic strategy for osteosarcoma. By targeting key cancer-related pathways, including apoptosis induction and cell cycle arrest, capsaicin may serve as a valuable adjunct or alternative to existing treatments. However, the study's limitations include the lack of *in vivo* validation, which is crucial to assess the bioavailability, efficacy, and safety of capsaicin in a whole organism. Future studies involving animal models are necessary to confirm the therapeutic potential of capsaicin and its ability to effectively treat osteosarcoma.

5. Future Perspectives

Further investigation of capsaicin's anticancer potential through comprehensive mechanistic research and sophisticated preclinical evaluations is future option for this study. A thorough grasp of its selective cytotoxicity can be obtained by extending *in vitro* research to include more osteosarcoma cell lines and healthy osteoblasts. Furthermore, to assess capsaicin's pharmacokinetics, bioavailability, and therapeutic effectiveness in a physiological context, *in vivo* models ought to be utilised. Capsaicin's stability, solubility, and targeted distribution to osteosarcoma cells may be improved by structural alterations made using analogue synthesis and nanoformulation techniques, which could also lessen any potential off-target effects. Further molecular docking and molecular dynamics simulations are required to improve its binding affinity and anticipate possible changes for enhanced drug-likeness, according to the *in-silico* interaction with 6T2W. Additionally, combining capsaicin with already available chemotherapeutic drugs may reveal potential synergistic effects that improve treatment efficacy and decrease drug resistance. Validating these results should be the main goal of future clinical trials and translational studies, which will ultimately aid in the creation of capsaicin-based treatments for the treatment of osteosarcoma.

6. Conclusion

This study successfully isolated and characterised capsaicin from *C. annuum* var. Cayenne, revealing its potent anticancer properties through GC-MS profiling, which identified several bioactive compounds. The anticancer activity of capsaicin was demonstrated on MG63 osteosarcoma cells, where it inhibited

cell proliferation and induced apoptosis. Molecular docking analysis further highlighted capsaicin's strong binding affinity with cancer-related targets, supporting its potential as a cancer therapeutic. Capsaicin's ability to target multiple cancer pathways underscores its promise as an effective agent against osteosarcoma. Future research should focus on *in vivo* studies to validate its efficacy and safety, as well as explore its potential in combination with existing treatments for enhanced therapeutic outcomes.

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8. Author Contribution

Kaniga Pandi – writing, original draft, Jubilee Ramasamy – data curation, Kavitha Kasimayan - software, Pradhosh Sakthivel – formal analysis, Rajeshkumar Thirupathi - conceptualization, Kadir Aravindhan – writing, review and editing, Hari Kumaravel – formal analysis, Binoy Varghese Cheriyan – supervision. All authors have read and agreed to the published version of the manuscript.

9. References

1. Bharadwaj N, Manimuthu MS, Vimal S, Radhakrishnan N. Evaluation of *in vitro* anti-cancer activity of methanolic leaf extract of phoenix pusilla on colon cancer cell line. J Pharm Bioallied Sci. 2024; 16(Suppl 2):S1181-S1185. https://doi.org/10.4103/jpbs.jpbs_522_23
2. Kumar V, Zahiruddin QS, Jena D, Ballal S, Kumar S, Bhat M, *et al.* Understanding current trends and incidence projections of prostate cancer in India: A comprehensive analysis of national and regional data from the global burden of disease study (1990-2021). Cancer Epidemiol. 2025; 94:102719. https://doi.org/10.1016/j.canep.2024.102719
3. Padmapriya A, Preetha S, Selvaraj J, Sridevi G. Effect of *Carica papaya* seed extract on IL-6 and TNF-α in human lung cancer cell lines-an *in vitro* study. Res J Pharm Technol. 2022; 15(12):5478-5482.
4. Prithiksha N, Priyadharshini R. *In vitro* molecular mechanisms of anticancer activity of stevioside in human osteosarcoma cell lines (Sarcoma Osteogenic). Contemp Clin Dent. 2024; 15(3):198-201. https://doi.org/10.4103/ccd.ccd_429_23
5. Kannan N, Ramalingam K, Ramani P, Krishnan M. Exploring prevalence trends of jaw bone pathologies: A three-year institutional study. Cureus. 2024; 16(5). https://doi.org/10.7759/cureus.60574
6. Hagelstein-Rotman M, Meier ME, Majoor BC, Cleven AH, Dijkstra PD, Hamdy NA, *et al.* Increased prevalence of malignancies in Fibrous Dysplasia/McCune-Albright Syndrome (FD/MAS): Data from a National Referral Center and the Dutch National Pathology Registry (PALGA). Calcif Tissue Int. 2021; 108:346-353. https://doi.org/10.1007/s00223-020-00780-6
7. Mirabello L, Pfeiffer R, Murphy G, Daw NC, Patiño-Garcia A, Troisi RJ, *et al.* Height at diagnosis and birth-weight as risk factors for osteosarcoma. Cancer Causes Control. 2011; 22:899-908. https://doi.org/10.1007/s10552-011-9763-2
8. Tsiambas E, Fotiades PP, Sioka C, Kotrotsios D, Gkika E, Fotopoulos A, *et al.* Novel molecular and metabolic aspects in osteosarcoma. J Buon. 2017; 22(6):1595-1598.
9. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: Current treatment and a collaborative pathway to success. J Clin Oncol. 2015; 33(27):3029-3035. https://doi.org/10.1200/jco.2014.59.4895
10. Das AP, Agarwal SM. Recent advances in the area of plant-based anti-cancer drug discovery using computational approaches. Mol Divers. 2024; 28(2):901-925. https://doi.org/10.1007/s11030-022-10590-7
11. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, *et al.* The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins. 2019; 11(11):656. https://doi.org/10.3390/toxins11110656
12. Radhakrishnan N, Vasantha S, Pandurangan AK (editors). Pharmacological benefits of natural agents. IGI Global; 2023.
13. Al Aboody MS, Mickymaray S. Anti-fungal efficacy and mechanisms of flavonoids. Antibiotics. 2020; 9(2):45. https://doi.org/10.3390/antibiotics9020045
14. Adamski Z, Blythe LL, Milella L, Bufo SA. Biological activities of alkaloids: From toxicology to pharmacology. Toxins. 2020; 12(4):210. https://doi.org/10.3390/toxins12040210
15. Uddandrao VS, Eraniappan S, Ramajayam AB, Singaravel S, Roy A, Parim BN, *et al.* Hydroxycitric acid and capsaicin combination alleviates obesity-induced testicular apoptosis, oxidative stress and inflammation. Syst Biol Reprod Med. 2024; 70(1):20-37. https://doi.org/10.1080/19396368.2024.2306403
16. Alasmary FA, Awaad AS, Kamal M, Alqasoumi SI, Zain ME. Antitumor activity of extract and isolated compounds from *Drechslera rostrata* and *Eurotiumtonophilum*. Saudi

Pharm J. 2018; 26(2):279-285. https://doi.org/10.1016/j.jsps.2017.11.011
17. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakri MM. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi JBiol Sci. 2018; 25(2):361-366. https://doi.org/10.1016/j.sjbs.2017.02.004
18. Pickersgill B. The domestication of chili peppers. The domestication and exploitation of plants and animals, Routledge; 2017: 443-450
19. Johnson Jr W. Final report on the safety assessment of *Capsicum annuum* extract, *Capsicum annuum* fruit extract, *Capsicum annuum* resin, *Capsicum annuum* fruit powder, *Capsicum frutescens* fruit, *Capsicum frutescens* fruit extract, *Capsicum frutescens* resin, and *Capsaicin*. Int JToxicol. 2007. https://doi.org/10.1080/10915810601163939
20. Jang JJ, Devor DE, Logsdon DL, Ward JM. A 4-week feeding study of ground red chilli (*Capsicum annuum*) in male B6C3F1 mice. Food Chem Toxicol. 1992; 30(9):783-787. https://doi.org/10.1016/0278-6915(92)90080-5
21. Kim JP, Park JG, Lee MD, Han MD, Park ST, Lee BH, Jung SE. Co-carcinogenic effects of several Korean foods on gastric cancer induced by N-methyl-N'-nitro-N-nitrosoguanidine in rats. Jap JSurg. 1985; 15:427-437. https://doi.org/10.1007/bf02470087
22. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. Cold spring harbor protocols. 2018; 2018(6):pdb-rot095505. https://doi.org/10.1101/pdb.prot095505
23. Odey MO, Iwara IA, Udiba UU, Johnson JT, Inekwe UV, Asenye ME, *et al.* Preparation of plant extracts from indigenous medicinal plants. Int J Sci Technol. 2012; 1(12):688-692.
24. Awasthi DN, Singh BP. Isolation and identification of capsaicin and allied compound in chilli. Proc/Ind Acad Sci. 1973; 77:196-201.
25. Ullucci PA, Cadoret R, Stasiowski PD, Martin HF. A comprehensive GC/MS drug screening procedure. J AnalytToxicol. 1978; 2(2):33-38.
26. Huey R, Morris GM, Forli S. Using AutoDock 4 and AutoDock vina with AutoDockTools: A tutorial. The Scripps Res Inst Mol Graph Lab. 2012; 10550(92037):1000.
27. Liu J, Yao Q, Peng Y, Dong Z, Tang L, Su X, *et al.* Identification of small-molecule inhibitors for osteosarcoma targeted therapy: Synchronizing *in silico*, *in vitro*, and *in vivo* analyses. Front Bioeng Biotechnol. 2022; 10:921107. https://doi.org/10.3389/fbioe.2022.921107
28. Jaghoori MM, Bleijlevens B, Olabarriaga SD. 1001 Ways to run AutoDock Vina for virtual screening. J Computer-aided Mol Design. 2016; 30:237-249. https://doi.org/10.1007/s10822-016-9900-9
29. Agnihotry S, Pathak RK, Srivastav A, Shukla PK, Gautam B. Molecular docking and structure-based drug design. Comput Aided Drug Des. 2020:115-131.
30. Cui H, Ali MY, Goyal P, Zhang K, Loh JY, Trybus KM, *et al.* Coiled-coil registry shifts in the F684I mutant of Bicaudal D result in cargo-independent activation of dynein motility. Traffic. 2020; 21(7):463-478. https://doi.org/10.1111/tra.12734
31. Bank PD. Protein data bank. Nature New Biol. 1971; 233(223):10-38.
32. Ragoza M, Masuda T, Koes DR. Generating 3D molecules conditional on receptor binding sites with deep generative models. Chem Sci. 2022; 13(9):2701-2713. https://doi.org/10.1039/d1sc05976a
33. Mukherjee S, Balius TE, Rizzo RC. Docking validation resources: Protein family and ligand flexibility experiments. J Chem InformModel. 2010; 50(11):1986-2000. https://doi.org/10.1021/ci1001982
34. Xiong Y, Yang HJ, Feng J, Shi ZL, Wu LD. Effects of alendronate on the proliferation and osteogenic differentiation of MG-63 cells. J Int Med Res. 2009; 37(2):407-416. https://doi.org/10.1177/147323000903700216
35. Lajeunesse D, Frondoza C, Schoffield B, Sacktor B. Osteocalcin secretion by the human osteosarcoma cell line MG-63. J Bone Mineral Res. 1990; 5(9):915-922. https://doi.org/10.1002/jbmr.5650050904
36. Suresh P, Xavier AS, Karthik VP, Punnagai K. Anticancer activity of *Cissus quadrangularis* L. methanolic extract against MG63 human osteosarcoma cells - An *in-vitro* evaluation using cytotoxicity assay. Biomed Pharmacol J. 2019; 12(2):975-980.
37. Adetunji TL, Olawale F, Olisah C, Adetunji AE, Aremu AO. Capsaicin: A two-decade systematic review of global research output and recent advances against human cancer. Front Oncol. 2022; 12:908487. https://doi.org/10.3389/fonc.2022.908487
38. Chen M, Xiao C, Jiang W, Yang W, Qin Q, Tan Q, *et al.* Capsaicin inhibits proliferation and induces apoptosis in breast cancer by down-regulating FBI-1-mediated NF-κB pathway. Drug Design, Development and Therapy. 2021:125-140. https://doi.org/10.2147/dddt.s269901
39. Chen M, Xiao C, Jiang W, Yang W, Qin Q, Tan Q, *et al.* Capsaicin inhibits proliferation and induces apoptosis in breast cancer by down-regulating FBI-1-mediated NF-κB pathway. Drug Design Develop Therapy. 2021:125-140. https://doi.org/10.2147/dddt.s269901
40. Liu J, Yao Q, Peng Y, Dong Z, Tang L, Su X, *et al.* Identification of small-molecule inhibitors for osteosarcoma targeted therapy: Synchronizing *in silico*, *in vitro*, and *in vivo* analyses. Front Bioeng Biotechnol. 2022; 10:921107. https://doi.org/10.3389/fbioe.2022.921107

41. Lal B, Kamboj A, Singh VP, Singh BP, Singh AP, Mishra SK. Phytochemical and biological properties of domesticated Capsicum species: A review. Intl J Plant Sci. 2023; 35:427-437.
42. Ledgerwood EC, Morison IM. Targeting the apoptosome for cancer therapy. Clin Cancer Res. 2009; 15(2):420-424. https://doi.org/10.1158/1078-0432.ccr-08-1172
43. Gimenez-Bonafe P, Tortosa A, Perez-Tomas R. Overcoming drug resistance by enhancing apoptosis of tumor cells. Curr Cancer Drug Targets. 2009; 9(3):320-340. https://doi.org/10.2174/156800909788166600
44. Bley K, Boorman G, Mohammad B, McKenzie D, Babbar S. A comprehensive review of the carcinogenic and anticarcinogenic potential of capsaicin. ToxicolPathol. 2012; 40(6):847-873. https://doi.org/10.1177/0192623312444471
45. Lee YS, Kang YS, Lee JS, Nicolova S, Kim JA. Involvement of NADPH oxidase-mediated generation of reactive oxygen species in the apototic cell death by capsaicin in HepG2 human hepatoma cells. Free Radic Res. 2004; 38(4):405-412. https://doi.org/10.1080/10715760410001665262
46. Lee YS, Nam DH, Kim JA. Induction of apoptosis by capsaicin in A172 human glioblastoma cells. Can Lett. 2000; 161(1):121-130. https://doi.org/10.1016/s0304-3835(00)00608-x
47. Federman N, Bernthal N, Eilber FC, Tap WD. The multidisciplinary management of osteosarcoma. Curr Treat OptionsOncol. 2009; 10:82-93. https://doi.org/10.1007/s11864-009-0087-3
48. De Luna-Bertos E, Ramos-Torrecillas J, García-Martínez O, Díaz-Rodríguez L, Ruiz C. Effect of aspirin on cell growth of human MG-63 osteosarcoma line. Sci World J. 2012; 2012(1):834246. https://doi.org/10.1100/2012/834246
49. Lin KL, Chi CC, Lu T, Tseng LL, Wang JL, Lu YC, *et al*. Effect of sertraline on [Ca2+]i and viability of human MG63 osteosarcoma cells. Drug Chem Toxicol. 2013; 36(2):231-240.
50. Jin S, Shen JN, Wang J, Huang G, Zhou JG. Oridonin induced apoptosis through Akt and MAPKs signaling pathways in human osteosarcoma cells. Cancer Biol Therap. 2007; 6(2):261-268. https://doi.org/10.4161/cbt.6.2.3621
51. Zhang R, Humphreys I, Sahu RP, Shi Y, Srivastava SK. *In vitro* and *in vivo* induction of apoptosis by capsaicin in pancreatic cancer cells is mediated through ROS generation and mitochondrial death pathway. Apoptosis. 2008; 13:1465-1478. https://doi.org/10.1007/s10495-008-0278-6
52. Mori A, Lehmann S, O'Kelly J, Kumagai T, Desmond JC, Pervan M, *et al*. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Cancer Res. 2006; 66(6):3222-3229. https://doi.org/10.1158/0008-5472.can-05-0087
53. Bai H, Li H, Zhang W, Matkowskyj KA, Liao J, Srivastava SK, *et al*. Inhibition of chronic pancreatitis and pancreatic intraepithelial neoplasia (panin) by capsaicin in lsl-kras g12d/pdx1-cre mice. Carcinogenesis. 2011; 32(11):1689-1696. https://doi.org/10.1093/carcin/bgr191