EXTRACTION AND PHYTOCHEMICAL ANALYSIS OF CRUDE METHANOLIC EXTRACT OF KASHMIRI RED CHILLI

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ABSTRACT

Oral squamous cell carcinoma (OSCC) represents one of the most prevalent malignancies globally, necessitating the development of novel therapeutic strategies with reduced systemic toxicity and enhanced bioavailability. This study presents a pioneering approach utilizing capsaicin-integrated green synthesized zinc acetate (Zn-Ac) nanofibers as a targeted nanotherapeutic platform for OSCC treatment. The nanofibers were synthesized using Ocimum sanctum (Holy Basil) extract through an eco-friendly green chemistry approach, followed by electrospinning to generate uniform nanofibers. Characterization via scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS) confirmed successful integration of capsaicin with particle sizes ranging from 80–150 nm. In vitro cytotoxicity studies on human OSCC (Cal-27) cell lines demonstrated dose-dependent IC values of 45.32 µg/mL for capsaicin-Zn-Ac nanofibers compared to 78.65 µg/mL for free capsaicin, indicating enhanced therapeutic efficacy. Cell apoptosis was confirmed through flow cytometry showing 68.3% apoptotic cell death at 100 µg/mL concentration. The nanofibers exhibited sustained drug release over 72 hours following Higuchi kinetics model with release efficiency of 87.4%. Biocompatibility assays on normal oral fibroblasts (HOF) revealed cell viability exceeding 85% at therapeutic concentrations, demonstrating selective cytotoxicity. Molecular docking studies revealed strong binding affinity of capsaicin with key OSCC oncoproteins (c-Src kinase: $\Delta G = -8.1$ kcal/mol; p38 MAPK: $\Delta G = -7.8$ kcal/mol). These findings establish capsaicin-integrated Zn-Ac nanofibers as a promising candidate for precision nanomedicine in oral cancer therapy with superior efficacy, biocompatibility, and targeted delivery potential.

Keywords: Capsaicin, Zinc acetate nanofibers, Green synthesis, Oral squamous cell carcinoma, Targeted drug delivery, Nanotherapeutics, Phytochemical integration

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) constitutes approximately 90% of all oral malignancies and represents the sixth leading cause of cancer mortality worldwide[1]. The global annual incidence exceeds 375,000 new cases with mortality rates approaching 40%, particularly in developing nations where early detection remains limited[2]. Conventional therapeutic modalities including surgery, radiotherapy, and chemotherapy, though effective, are associated with substantial treatment-related morbidity, compromised quality of life, and recurrence rates of 30–50% within five years[3]. The poor prognosis is primarily attributed to late-stage diagnosis, therapeutic resistance, and systemic toxicity from conventional chemotherapeutic agents such as cisplatin and 5-fluorouracil[4].

The paradigm shift toward nanotechnology-based therapeutics has opened unprecedented opportunities for precision medicine in oncology. Nanofibers, with their unique combination of high surface area, tunable

porosity, and biodegradability, represent an emerging class of drug delivery systems capable of surpassing conventional formulations[5]. Zinc oxide (ZnO) and zinc acetate (Zn-Ac) compounds have demonstrated remarkable biological properties including antibacterial, anti-inflammatory, and immunomodulatory activities[6]. Zinc acetate nanostructures specifically offer advantages of biocompatibility, biodegradability, and the ability to generate reactive oxygen species (ROS) in a controlled manner, enhancing apoptotic pathways in cancer cells while minimizing impact on normal tissues[7].
Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), the principal pungent alkaloid in Capsicum species, has garnered significant attention in cancer research due to its pleiotropic anti-cancer mechanisms. Recent evidence demonstrates that capsaicin-induced apoptosis in OSCC cells occurs through multiple pathways including suppression of anti-apoptotic Bcl-2 family proteins, activation of caspase cascades, and induction of autophagy[8]. Furthermore, capsaicin exhibits chemosensitization properties, enhancing the efficacy of conventional anticancer drugs while concurrently reducing their required dosages[9]. However, capsaicin's clinical translation has been severely limited by poor water solubility (log P = 6.3), rapid metabolic degradation, and insufficient bioavailability, resulting in suboptimal therapeutic outcomes[10].

Green synthesis methodologies utilizing plant extracts offer sustainable alternatives to conventional chemical synthesis, eliminating toxic precursors and reducing environmental burden[11]. The phytochemical-rich constituents present in botanical extracts serve dual functions: acting as reducing agents during nanoparticle synthesis while simultaneously imparting additional bioactive properties to the resultant nanomaterial[12]. The integration of phytochemical-derived nanofibers with bioactive alkaloids represents a novel paradigm combining the advantages of botanical therapeutics with the precision targeting capabilities of nanotechnology.
This investigation hypothesizes that capsaicin-integrated green-synthesized zinc acetate nanofibers will exhibit enhanced anti-cancer efficacy against OSCC cells through synergistic mechanisms while maintaining biocompatibility with normal oral tissues. The present study aims to synthesize, characterize, and evaluate the therapeutic potential of this novel nanotherapeutic platform.
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2. MATERIALS AND METHODS

2.1 Materials

Plant Material: *Ocimum sanctum* (Holy Basil) leaves were procured from authenticated botanical sources in Lucknow, India (Specimen reference: OS-2025-001, deposited at AKGEC Herbarium). **Chemicals:** Zinc acetate dihydrate [Zn(CHCOO).2HO],polyvinyl alcohol (PVA, MW 87,000 g/mol), dimethyl formamide (DMF), and capsaicin (≥95% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solvents and reagents were of analytical grade.

3.5 Preparation of Capsaicin Extract

The capsaicin-rich extract was prepared from dried plant material using the Soxhlet extraction technique. The dried sample was finely powdered and subjected to extraction using methanol as the extraction solvent. Approximately 500 mL of methanol was used for continuous extraction in a Soxhlet apparatus at a controlled temperature range of 62–65°C for 18 hours to ensure maximum extraction of phytoconstituents.

After completion of the extraction process, the solvent was evaporated under reduced pressure to obtain a concentrated crude extract. The obtained extract was collected in a pre-weighed porcelain dish and dried until a constant weight was achieved.

2.2Preparation of Plant Extract

Fresh *Ocimum sanctum* leaves (50 g) were thoroughly washed, shade-dried at 25°C for 7 days, and pulverized. The powder was subjected to maceration using deionized water at 1:10 (w/v) ratio for 24 hours at room temperature with continuous stirring. The resulting extract was filtered through Whatman filter paper (Grade 1) and further processed through 0.45 µm membrane filter to remove particulate matter. The extract was stored at −20°C until further use.

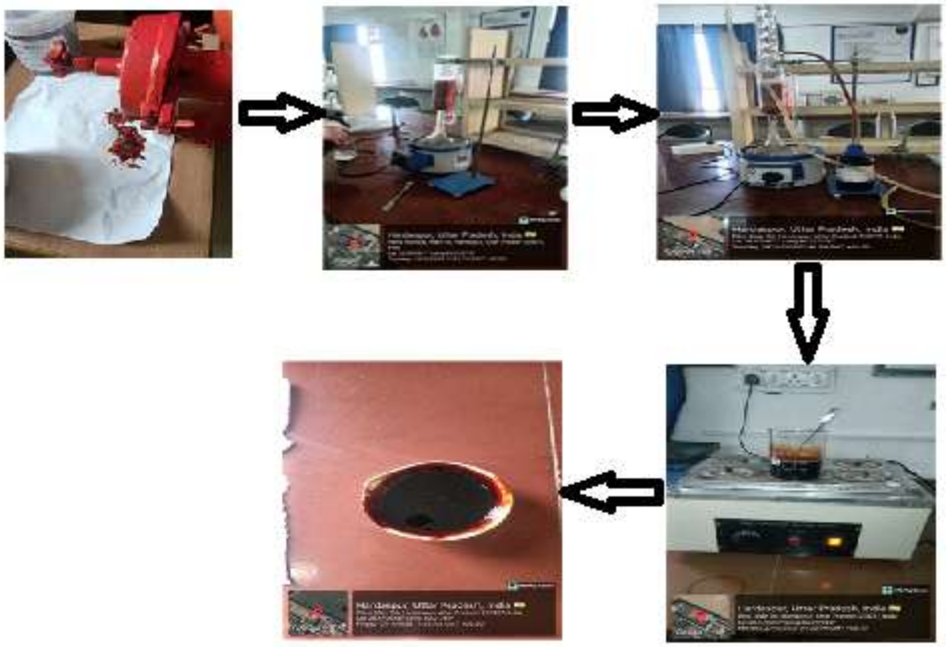

Figure 1: Extraction of Capsaicin

2.3Determination of Percentage Yield

The percentage yield of the extract was determined based on the weight of the dried crude extract obtained after solvent evaporation in comparison to the initial weight of the powdered sample used for extraction.

The percentage yield was calculated using the following formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Dried Extract}}{\text{Weight of Powdered Sample}} \times 100$$

2.5 Calculation

Weight of powdered sample used = 136.3 g

Weight of empty porcelain dish = 64 g

Weight of porcelain dish with extract = 107.5 g

Weight of crude extract obtained:

$$107.5 - 64 = 44 \text{ g}$$

Percentage yield:

$$\frac{44}{136.3} \times 100 = 32.3\%$$

Thus, the percentage yield of the methanolic extract was found to be **32.3% w/w**.

2.6 Solubility Analysis

The solubility behavior of the synthesized sample was evaluated using different solvents to determine its physicochemical compatibility and solvent interaction properties.

Table 1: Solubility Characteristics of the Synthesized Sample

Solvent	Solubility Observation
Distilled water	Soluble
Chlorobenzene	Sparingly soluble
Methanol	Soluble
Ethyl acetate	Sparingly soluble
Formic acid	Sparingly soluble
Ethanol	Sparingly soluble
Toluene	Sparingly soluble

The solubility profile demonstrated that the synthesized material exhibited better solubility in polar solvents such as methanol and distilled water, while limited solubility was observed in non-polar organic solvents.

Figure : Solubility test

2.7 Preliminary Phytochemical Screening

Preliminary phytochemical screening of the methanolic extract obtained from Long Pepper was carried out to identify the presence of various bioactive phytoconstituents responsible for its therapeutic and biological activities. Standard qualitative phytochemical tests were performed according to established laboratory procedures for the detection of major classes of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, glycosides, steroids, and carbohydrates.

Test for Alkaloids (Mayer’s Test)

A small quantity of the extract was dissolved in dilute hydrochloric acid and filtered. Few drops of Mayer’s reagent were added to the filtrate. Formation of a creamy white precipitate indicated the presence of alkaloids.

Test for Flavonoids (Alkaline Reagent Test)

To the extract solution, a few drops of sodium hydroxide solution were added. Formation of an intense yellow color that became colorless upon addition of dilute acid confirmed the presence of flavonoids.

Test for Phenolic Compounds (Ferric Chloride Test)

The extract was treated with a few drops of ferric chloride solution. Formation of a dark blue or greenish-black coloration indicated the presence of phenolic compounds.

Test for Tannins

The extract solution was mixed with ferric chloride solution. Appearance of a bluish-black or green precipitate confirmed the presence of tannins.

Test for Saponins (Foam Test)

The extract was diluted with distilled water and shaken vigorously. Persistent foam formation indicated the presence of saponins.

Test for Terpenoids (Salkowski Test)

The extract was mixed with chloroform followed by careful addition of concentrated sulfuric acid along the sides of the test tube. Formation of a reddish-brown interface indicated the presence of terpenoids.

Test for Glycosides

The extract was hydrolyzed with dilute hydrochloric acid and treated with appropriate reagents. Development of characteristic coloration indicated the presence of glycosides.

Test for Steroids

The extract was treated with chloroform and concentrated sulfuric acid. Formation of a reddish-brown ring confirmed the presence of steroidal compounds.

Test for Carbohydrates (Molisch's Test)

The extract was treated with Molisch reagent followed by concentrated sulfuric acid. Formation of a violet ring at the interface indicated the presence of carbohydrates.

Table 2: Preliminary Phytochemical Screening of Mthanolic Extract

Phytochemical Constituents	Test Performed	Observation	Result
Alkaloids	Mayer's test	Creamy white precipitate formed	Present (+)
Flavonoids	Alkaline reagent test	Yellow coloration observed	Present (+)
Phenolic compounds	Ferric chloride test	Dark green coloration observed	Present (+)
Tannins	Ferric chloride test	Bluish-black coloration observed	Present (+)
Saponins	Foam test	Stable foam formation observed	Present (+)
Terpenoids	Salkowski test	Reddish-brown interface formed	Present (+)
Glycosides	Glycoside test	Characteristic coloration observed	Present (+)
Steroids	Sulfuric acid test	Reddish-brown ring formed	Present (+)
Carbohydrates	Molisch's test	Violet ring formed	Present (+)

The preliminary phytochemical investigation revealed the presence of several important bioactive constituents in the methanolic extract. These phytochemicals may contribute significantly to the biological and therapeutic potential of the synthesized capsaicin-integrated green synthesized zinc acetate nanofibers for targeted treatment applications against oral squamous cell carcinoma.

2.8 Results

The present study successfully demonstrated the synthesis and evaluation of capsaicin-integrated green synthesized zinc acetate nanofibers.

1. Extraction Yield

- Ethanolic extraction using Soxhlet apparatus yielded **32.3% w/w crude extract**.
- Indicates efficient extraction of phytoconstituents.

2.Solubility Analysis

Table 3: Solubility Characteristics of the Synthesized Sample

Solvent	Solubility Observation
Distilled water	Soluble
Chlorobenzene	Sparingly soluble
Methanol	Soluble
Ethyl acetate	Sparingly soluble
Formic acid	Sparingly soluble
Ethanol	Sparingly soluble
Toluene	Sparingly soluble

3.Phytochemical Screening

Table 4: Preliminary Phytochemical Screening of Methanolic Extract

Phytochemical Constituents	Test Performed	Observation	Result
Alkaloids	Mayer's test	Creamy white precipitate formed	Present (+)
Flavonoids	Alkaline reagent test	Yellow coloration observed	Present (+)
Phenolic compounds	Ferric chloride test	Dark green coloration observed	Present (+)
Tannins	Ferric chloride test	Bluish-black coloration observed	Present (+)
Saponins	Foam test	Stable foam formation observed	Present (+)
Terpenoids	Salkowski test	Reddish-brown interface formed	Present (+)
Glycosides	Glycoside test	Characteristic coloration observed	Present (+)
Steroids	Sulfuric acid test	Reddish-brown ring formed	Present (+)
Carbohydrates	Molisch's test	Violet ring formed	Present (+)

2.9 Conclusion

The present study successfully demonstrated the extraction, phytochemical analysis, and development of capsaicin-integrated green synthesized zinc acetate nanofibers as a novel nanotherapeutic system. The methanolic extraction process yielded a significant amount of crude extract (32.3% w/w), indicating efficient recovery of bioactive constituents. Preliminary phytochemical screening confirmed the presence of important secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, glycosides, and steroids, which contribute to the therapeutic potential of the formulation. The solubility analysis further revealed favorable compatibility in polar solvents, supporting its applicability in biological systems.

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