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Exploring the phytochemical constituents present in *Capsicum frutescens*: Isolation and their pharmacological evaluation

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Abstract
The current study focuses on the complete phytochemical analysis and pharmacological assessment of *Capsicum frutescens*, a plant of high medical and nutritional significance commonly employed in traditional medicine. The research involved the isolation, identification, and assessment of the bioactive constituents responsible for the therapeutic properties of the plant. Soxhlet apparatus-based methanolic extraction provided 3.18% w/w extractive value, representing a reasonable amount of bioactive content for scrutiny. Initial phytochemical screening confirmed the presence of a wide range of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, sterols, phenolics, carbohydrates, and proteins. These compounds are well-established for their pharmacological properties, specifically antioxidant, anti-inflammatory, and antimicrobial activities. Pharmacognostical analysis confirmed the identity and purity of the crude drug, with total ash at 8.67% and loss on drying at 16.13%, demonstrating acceptable levels of moisture and inorganic residues. Quantitative phytochemical analysis determined the total phenolic content of the methanolic extract to be 20 ± 0.06 µg/mL, indicating strong evidence of antioxidant polyphenols. Fluorescence analysis under UV and in various reagents further confirmed the chemical richness and authenticity of the plant. Sophisticated analytical methods were used to establish the phytoconstituent composition. High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) in combination characterized several prominent and minor constituents, including capsaicin, dihydrocapsaicin, nordihydrocapsaicin, caffeoylquinic acid derivatives, quercetin glycosides, acylated flavonoids, carotenoid esters (like capsanthin), and high molecular weight glycosylated lipids. The presence of these constituents emphasizes the plant's promise in oxidative stress-associated and metabolic disorders. The methanolic extract was tested for its pharmacological potential by evaluating antioxidant activity. In DPPH free radical scavenging activity, the extract showed 88.4% inhibition at 1000 µg/mL with an IC_{50} of 204.8 µg/mL, comparable to standard antioxidants like ascorbic acid and BHT. Hydrogen peroxide scavenging and reducing power assays further validated the marked antioxidant activity of the extract. Furthermore, the lactate dehydrogenase (LDH) enzyme assay showed potential cellular protection or metabolic stimulation, suggesting cytoprotective benefits. In brief, this research supports the traditional uses of *Capsicum frutescens* as an affluent source of therapeutically important phytochemicals with potent antioxidant activity. The synergistic combination of phytochemical diversity and pharmacological potency positions this plant at the center of future drug development and natural product-based therapeutic interventions. Isolation of individual compounds, their *in vivo* efficacy, and development of standardized preparations are recommended for further clinical application.

Keywords: *Capsicum frutescens*, DPPH, LDH, phytochemical, HPLC, antioxidant, pharmacological evaluation

Introduction
Capsicum frutescens, commonly known as bird's eye chili or African chili, is a perennial shrub belonging to the Solanaceae family, widely cultivated in subtropical and tropical regions for its culinary, medicinal, and nutritional value. This plant is distinguished by its small, intensely pungent fruits, which owe their characteristic heat to high concentrations of capsaicinoids, particularly capsaicin. Beyond its role in enhancing flavor and spice in global cuisines, *Capsicum frutescens* has been integral to traditional medicine systems for centuries, where it is employed to alleviate a variety of ailments including pain, inflammation, digestive disorders, and respiratory issues.
The pharmacological versatility of *Capsicum frutescens* stems from its rich array of bioactive compounds. Capsaicin, the primary capsaicinoid, interacts with transient receptor potential vanilloid 1 (TRPV1) channels, leading to initial activation followed by desensitization of sensory neurons. This mechanism underpins its analgesic and anti-inflammatory effects,

making it a natural alternative for managing chronic pain conditions such as arthritis and neuropathy. Furthermore, capsaicin's ability to modulate inflammatory pathways by inhibiting enzymes like Cyclooxygenase (COX) and Lipoxygenase (LOX) highlights its potential in reducing pro-inflammatory mediators. In addition to pain relief, emerging evidence points to the plant's benefits in metabolic health. Capsaicin enhances thermogenesis, promotes fat oxidation, and improves insulin sensitivity, positioning *Capsicum frutescens* as a candidate for addressing obesity and type 2 diabetes. Its antioxidant properties, derived from phenolics, flavonoids, and carotenoids, combat oxidative stress, a key factor in chronic diseases like cardiovascular disorders and cancer. The plant's antimicrobial activity against bacteria, fungi, and viruses further supports its use in infection control, especially in the face of rising antibiotic resistance.

Historically, Capsicum species, including frutescens, originated in the Americas and spread globally post-Columbian exchange, integrating into diverse cultural and medicinal practices. In Ayurveda, it aligns with concepts of balancing doshas through its heating properties, while in Traditional Chinese Medicine, it is used to dispel cold and invigorate qi. Indigenous communities have long harnessed its fruits, leaves, and seeds for wound healing, fever reduction, and as a stimulant. With over 80% of the world's population relying on plant-based remedies, validating these traditional uses through scientific scrutiny is imperative, particularly given concerns over synthetic drug side effects and costs. The safety profile of *Capsicum frutescens*, when used judiciously, is favorable compared to synthetic analogs, though caution is advised in sensitive populations due to potential gastrointestinal irritation. This study aims to bridge traditional knowledge with modern analytical techniques, exploring the phytochemical diversity and pharmacological efficacy of *Capsicum frutescens* leaves to uncover its therapeutic potential in oxidative stress-related and metabolic disorders.

Historical and cultural significance

The utilization of medicinal plants like *Capsicum frutescens* dates back millennia, with archaeological evidence suggesting human reliance on botanicals for health as early as 60,000 years ago. Ancient civilizations in Egypt, Mesopotamia, China, and India documented extensive herbal pharmacopeias. The Ebers Papyrus (circa 1500 BC) lists over 800 remedies, while Chinese texts like the Nei Ching and Indian Ayurvedic scriptures such as Charak Samhita (1000 BC) describe thousands of plant-based formulations emphasizing prevention over cure.

Ayurveda's eight branches encompassing internal medicine, surgery, paediatrics, toxicology, and rejuvenation illustrate a holistic approach where plants like chili peppers balance bodily humors. Hippocrates' dictum, "Let food be thy medicine," echoes this philosophy, viewing illness as an imbalance remedied by natural substances. Culturally, *Capsicum frutescens* embodies ancestral wisdom in indigenous healing, contributing to biodiversity conservation and community health in regions where modern medicine is inaccessible.

Current landscape and drug discovery from plants

In contemporary healthcare, plants like *Capsicum frutescens* supply bioactive compounds for 25% of prescriptions in developed nations and up to 90% of global pharmaceuticals. Advances in NMR, LC-MS/MS, and biotechnology have expedited compound isolation, though challenges like intellectual property and sustainable sourcing persist under frameworks like the Convention on Biological Diversity.

Initiatives in countries like India integrate traditional systems with modern validation, fostering drug discovery for conditions where synthetic options fall short.

Significance of medicinal plants and rationale for evaluation

Medicinal plants underpin global health economies, providing livelihoods and cultural continuity. Threats like overharvesting necessitate conservation. Phytochemical and pharmacological assessments identify bioactives, validate traditions, and standardize products, driving innovation in nutraceuticals and therapeutics.

Throwback in study

Recent investigations into *Capsicum frutescens* underscore its pharmacological promise, driven by capsaicinoids like capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, alongside carotenoids and saponins. These compounds exhibit antioxidant capabilities by scavenging free radicals, anti-inflammatory effects via TRPV1 modulation, and antimicrobial action against pathogens including bacteria and fungi. Studies have authenticated antidiabetic properties through improved glucose metabolism, cardiovascular benefits via vasodilation and lipid regulation, thrombolytic activity for clot dissolution, and anticancer potential by inducing apoptosis in tumor cells. Biosynthesis pathways in plants highlight capsaicin's natural production, while green synthesis methods address industrial scalability for applications in pain management, obesity control, and food preservation. Comparative analyses with related species like *Capsicum annuum* and chinense reveal genotype-dependent variations in phenolic, flavonoid, and capsaicinoid content, influencing antioxidant and enzyme-inhibitory activities relevant to diabetes management. Ethanolic extracts often outperform aqueous ones in yield and bioactivity, with ripe fruits showing higher phenolic levels.

In silico docking suggests inhibitory effects on receptors like estrogen alpha, supporting anticancer uses. Antimicrobial evaluations of hexane and ethanol extracts demonstrate efficacy against resistant strains, reinforcing traditional applications.

Overall, these findings affirm *Capsicum frutescens* as a multifaceted therapeutic agent, with ongoing advancements in nanoencapsulation enhancing delivery for clinical efficacy.

Materials and Methods

Plant collection and authentication

Fresh leaves of *Capsicum frutescens* were collected in February 2025 from the Botanical Garden of India and authenticated (Ref no: 2805250055712) by Dr. Vinay Ranjan, Department of Botany, Botanical Garden of India, Uttar Pradesh, India.

Extraction

1.0 kg of semi-dried, coarsely crushed leaves was extracted using 90% methanol in a Soxhlet apparatus, yielding 31.80 g of dark brown residue (3.18% w/w).

Preliminary phytochemical screening

Qualitative tests for alkaloids (Dragendorff's, Mayer's,

Wagner's, Murexide), carbohydrates (Molisch, Fehling), glycosides (Keller-Killani, Legal, Baljet), phenolics (ferric chloride, lead acetate, gelatin), flavonoids (ammonia, Shinoda), proteins/amino acids (Millon's, Xanthoprotein, Ninhydrin, Piotrowski's), saponins (foam), sterols (Salkowski, Liebermann-Burchard), terpenoids (Noller's), and resins were performed.

Pharmacognostical investigation
Fluorescence analysis under UV (240 nm, 360 nm) and reagents; ash values (total, acid-insoluble, water-soluble); loss on drying at 105°C.

Quantitative phytochemical determination
Total phenolics (Folin-Ciocalteu, gallic acid standard); flavonoids (AlCl3, ferulic acid standard); saponins (vanillin-sulfuric acid); alkaloids (acetic acid-ethanol extraction).

Chromatographic and spectroscopic profiling
TLC (silica gel G); HPLC (Agilent 1100, C18 column, chloroform: methanol 8:2); MS (Agilent); NMR (Bruker, CDCl3); IR (Alpha Bruker, KBr pellet) for standards like gallic acid.

***In vitro* antioxidant activity**
DPPH scavenging (517 nm); H_2O_2 scavenging (230 nm); reducing power (700 nm); standards: ascorbic acid, BHT.

LDH enzyme assay
Assay buffer (Tris-HCl pH 7.5), NAD+, pyruvate; absorbance at 340 nm.

Results

Extraction yield and phytochemical screening
Methanolic extract yield: 3.18% w/w. All tested phytoconstituents were present, confirming the presence of diverse secondary metabolites essential for bioactivity.

Table 1: Preliminary phytochemical screening of the extract

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's	+
Carbohydrates	Molisch	+
Glycosides	Keller-Killani	+
Phenolics	Ferric chloride	+
Flavonoids	Shinoda	+
Proteins/Amino acids	Millon's	+
Saponins	Foam	+
Sterols	Salkowski	+
Terpenoids	Noller's	+
Resins	Turbidity	+

(+ = Present)

Pharmacognostical parameters
Fluorescence analysis revealed characteristic emissions, aiding authentication. Total ash: 8.67%; acid-insoluble ash: 3.33%; water-soluble ash: 5.33%; loss on drying: 16.13%, indicating suitable quality for further analysis.

Quantitative phytoconstituents
Total phenolics: 20 ± 0.06 µg/mL (gallic acid equivalent), underscoring strong antioxidant potential.

Chromatographic and spectroscopic data
HPLC displayed multiple peaks (5-25 min), with MS confirming capsaicin (m/z 306), dihydrocapsaicin (m/z 308), and others. NMR (^{1}H at 7.05 ppm for gallic acid) and IR (3500-2500 cm^{-1} for OH) validated structures.

Antioxidant activity
DPPH: 88.4% inhibition at 1000 µg/mL, IC_{50} 204.8 µg/mL.
H_2O_2 scavenging: IC_{50} 19.20 µg/mL.
Reducing power: Dose-dependent increase, akin to ascorbic acid and BHT.

Table 2: DPPH radical scavenging activity and IC_{50} value of the extract

Concentration (µg/mL)	DPPH % inhibition (Extract)	IC_{50} (µg/mL)
1000	88.4	204.8

LDH assay
Demonstrated cytoprotective effects, suggesting metabolic enhancement and cellular protection.

Discussion

The methanolic extract of *Capsicum frutescens* leaves exhibited a robust phytochemical profile, aligning with its traditional applications in pain relief, inflammation reduction, and metabolic support. The identified capsaicinoids, flavonoids, and carotenoids correlate with potent antioxidant activities observed in DPPH, H2O2, and reducing power assays, comparable to established standards. These results resonate with broader findings where capsaicinoids demonstrate antidiabetic effects by enhancing glucose uptake, cardiovascular benefits through vasodilation, and anticancer potential via apoptosis induction. Antimicrobial evaluations further reveal efficacy against resistant microbes, while green synthesis advancements suggest scalable production for therapeutic formulations.
The LDH assay's indication of cytoprotection complements the plant's role in oxidative stress mitigation, potentially extending to neurodegenerative and hepatic disorders. Variations in bioactivity across Capsicum species highlight the need for genotype-specific studies, with ethanolic extracts often yielding superior results.
This investigation validates *Capsicum frutescens* as a versatile natural resource, with implications for integrative medicine and sustainable drug development.

Conclusion

The present study, "Exploring the Phytochemical Constituents Present in *Capsicum frutescens*, Isolation and Their Pharmacological Evaluation", investigates the chemical profile and biological potential of *C. frutescens*. Methanolic extraction (3.18% w/w yield) revealed diverse secondary metabolites including alkaloids, flavonoids, glycosides, terpenoids, saponins, phenolics, tannins, sterols, and proteins. Pharmacognostic parameters (LOD 16.13%, total ash 8.67%) confirmed quality, while UV fluorescence and HPLC, MS, NMR, and IR analyses identified major compounds such as capsaicin, dihydrocapsaicin, quercetin glycosides, capsanthin, ferulic acid, and gallic acid. Antioxidant evaluation showed strong activity: 88.4% DPPH scavenging (IC_{50} 204.8 µg/mL), potent hydrogen peroxide scavenging (IC_{50} 19.20 µg/mL), and high reducing power. LDH assays suggested cytoprotective potential. These results validate traditional uses of *C. frutescens* and

highlight its potential in managing oxidative stress, inflammation, and metabolic disorders, supporting its development as a phytotherapeutic resource.

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