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Research Article

Phytochemical and Pharmacological Evaluation of *Capsicum Annuum*

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ABSTRACT

Capsicum Annuum is a plant species of high gastronomic value, appreciated for its pungent taste and potential medicinal properties. This study explores its phytochemical composition and pharmacological activities to evaluate its health benefits and therapeutic potential. The total extract yield was 2.64%, indicating a rich presence of bioactive compounds. Systematic extraction revealed key phytochemicals such as alkaloids, flavonoids, glycosides, saponins, and terpenoids each linked to various health-promoting and disease-preventing effects. These methods ensured accurate structural identification and reliable, reproducible results. The extract showed 17.98% scavenging activity against hydrogen peroxide, suggesting potential in combating oxidative stress a factor in diseases like cancer, cardiovascular disorders, and neurodegeneration. Future studies should focus on elucidating the mechanisms of action and specific biological pathways influenced by these compounds, facilitating the development of targeted therapies and nutritional supplements.

INTRODUCTION

Capsicum annuum is particularly rich in its phytochemical content, which comprises capsaicin, capsanthin, flavonoids, carotenoids, and vitamins A, C, and E. Among them, capsaicin although generally lower in content than *Capsicum annuum* is a critical determinant of its pharmacological actions. Capasaicin's effects have been validated through studies on pain relief, anti-inflammatory activities, antioxidant functions, and even antimicrobial activity. Its effects are mediated through its action on transient receptor potential vanilloid 1 (TRPV1) receptors, which regulate nociception (perception of pain), inflammation, and thermoregulation. Capsaicin-induced desensitization of TRPV1 channels provides a natural method of controlling chronic pain, osteoarthritis, and inflammatory disorders [1-8].

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Consistent with the World Health Organization's promotion of the inclusion of traditional and complementary medicine within formal health systems, *Capsicum annuum* has regained attention based on its traditional applications and contemporary therapeutic potential. Traditional cultures within the Americas have used different forms of *Capsicum annuum* preparations to treat disorders like digestive issues, respiratory ailments, and infections. These ethnobotanical traditions form the basis of contemporary pharmacognosy, yielding useful leads for pharmaceutical and nutraceutical uses [9-12].

As consumers increasingly become suspicious of side effects linked with man-made pharmaceuticals, plant remedies such as *Capsicum annuum* present perceived safety, cultural acceptability, and efficacy. Most importantly, more than 80% of the world's population depends on traditional medicine for basic health care needs, which shows immense global dependence on botanicals as alternatives or supplements to allopathic treatments [13-18].

PLANT PROFILE

CAPSICUM ANNUUM

It commonly referred to as sweet pepper, bell pepper, or hot pepper (depending on the cultivar), is a widely cultivated annual or perennial herbaceous plant belonging to the Solanaceae family[19]. Native to Central and South America, this versatile species has been domesticated and adapted across the globe, thriving in tropical, subtropical, and temperate climates. The plant generally reaches a height of 1 to 4 feet, with a bushy and branched structure. Its foliage consists of broad, ovate, dark green leaves, while the flowers are typically white and solitary, emerging from the leaf axils [20-26].

Fig – 1 – *Capscicum Annuum*

BOTANICAL CLASSIFICATION

- **Kingdom**: **Plantae**
- **Clade**: Angiosperms
- **Clade**: Eudicots
- **Order**: Solanales
- **Family**: Solanaceae
- **Genus**: *Capsicum*
- **Species**: *Capsicum annuum* L.

PHYTOCHEMICAL REVIEW

Capsaicinoids

- **Primary Compounds:** Capsaicin and dihydrocapsaicin are the major capsaicinoids responsible for the pungency in *Capsicum annuum*. These compounds activate the TRPV1 receptors, which are involved in pain perception [27].
- **Pharmacological Actions:** Capsaicinoids exhibit analgesic, anti-inflammatory, antioxidant, and anticancer activities. The analgesic properties make them suitable for topical pain relief, especially in conditions like arthritis and neuropathic pain.
- **Biosynthesis:** Capsaicinoids are synthesized in the placental tissues of the fruit, with their concentration increasing as the fruit matures.[28-31]

Carotenoids

- **Primary Compounds:** Beta-carotene, lutein, and zeaxanthin are prominent carotenoids in

Capsicum annuum, providing antioxidant properties and contributing to the red and orange hues of the ripe fruit.

- **Health Benefits:** Carotenoids support immune function, promote eye health, and help reduce the risk of chronic diseases such as cardiovascular disease [32].

Flavonoids

- **Primary Compounds:** Quercetin, luteolin, and apigenin are the main flavonoids found in *Capsicum annuum*. These compounds contribute to the plant's antioxidant, anti-inflammatory, and antimicrobial activities [33].
- **Biological Functions:** Flavonoids scavenge free radicals, reducing oxidative stress and inflammation. Their antimicrobial activity is particularly valuable in protecting the plant from microbial pathogens.
- **Health Implications:** Flavonoids in *Capsicum annuum* support cardiovascular health, improve immune response, and may provide protective effects against certain cancers.[34-36]

Vitamins and Minerals

- **Vitamin C:** *Capsicum annuum* is rich in ascorbic acid (vitamin C), an antioxidant that supports immune health, skin health, and aids in collagen synthesis.
- **Vitamin E:** Known for its antioxidant properties, vitamin E protects cell membranes from oxidative damage and plays a role in skin health [37].

Alkaloids and Other Secondary Metabolites

- **Minor Alkaloids:** Apart from capsaicinoids, other alkaloids such as piperidine and pyrazine derivatives may also be present in trace amounts, contributing to the plant's aroma and minor pharmacological properties.
- **Saponins and Tannins:** These compounds possess mild antimicrobial and antioxidant properties, adding to the overall protective effect of *Capsicum annuum* against pathogens. [38-41]

The phytochemical profile of *Capsicum annuum* provides a basis for its traditional and therapeutic applications. The synergistic effects of these bioactive compounds offer broad-spectrum health benefits, including pain relief, antioxidant protection, immune enhancement, and anti-inflammatory action.[42,43]

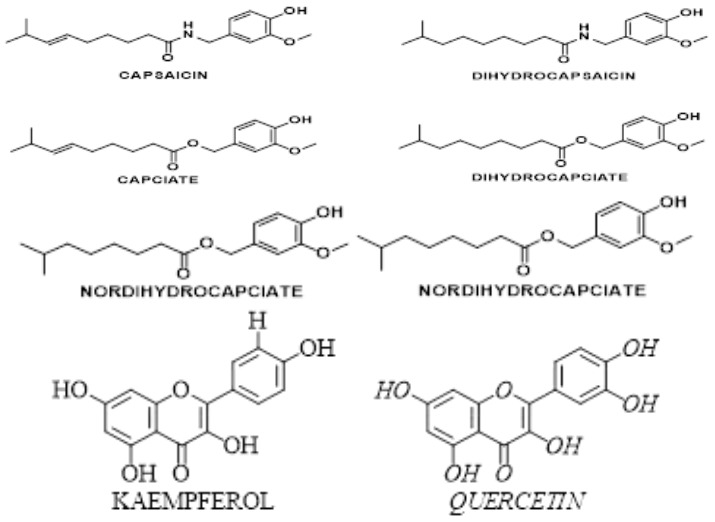

Figure 2. Structure of isolated phyto-constituents Capsicum annum.

MATERIAL AND METHOD

1. Collection and authentication of plant material

The fresh leaves of *Capsicum annuum* were collected in the month of February 20225 from the botanical garden of Botinical survey of India. The plant and specimen were collected by local and the specimens (ref no 1203250014687) were authenticated by Dr. Vinay Ranjan, Department of Botany, Botanical survey of India Uttar Pradesh, India.

2. Apparatus, Instruments, Chemicals and Drugs

- Merck Chemicals and Fischer scientific provided all the required chemicals and reagents, and a Single Pan Mettler weighing balance was used for all weighing operations.
- A Lambda Bio 20 Spectrophotometer was used to collect ultraviolet spectra in methanol. From Sisco Research Laboratory (SRL), Mumbai, India, we acquired ascorbic acid, ferrous sulphate, trichloro acetic acid, deoxyribose, and thio-barbituric acid.
- Spots were seen on silica gels G (Merck) used for TLC after being exposed to iodine vapours and UV light.
- Bruker NMR, Agilent mass spectrophotometer was used for the characterization.
- Quali-tech have provided the chemical required for the LDH assay.

3. Preparation of extract

Using a Soxhlet apparatus, 1.5 kg of shade-dried, coarsely crushed *Capsicum annuum* leaves was extracted with 95% methanol as the solvent. The methanolic extract after the extraction process was dried on an intense steam bath under reduced pressure, and 39.60 g of dark brown residue was obtained.

4. Preliminary phytochemical analysis

The crude plant extract was put to preliminary phytochemical screening in the search for all classes of bioactive constituents.

A) Tests for alkaloids

Following alkaloid screenings were performed on a sample of 5 ml methanolic extract on *Capsicum annuum* leaf extract.

a. Dragendorff's Test;

Filter paper impregnated with Dragendorff's reagent was applied over the extract. Bricked or reddish-brown color resulted as an affirmation of positive alkaloids screenings.

b. Mayer's Test (Potassium Mercuric Iodide Reagent)

When Mayer's reagent was added to the extract, a cream-coloured precipitate formed; thus, proving the existence of alkaloids.

c. Wagner's Test (Iodine-Potassium Iodide Reagent)

A few drops of Wagner's reagent when added to the test filtrate led to the formation of a brown flocculent precipitate, proving for the existence of alkaloids.

d. Murexide Test

When the reagent was applied a purple color reaction appeared. Thus, the presence of alkaloids was confirmed.

B) Test for Carbohydrates

a. Molisch test

Dilute, with distilled water up to a volume of 5 mL, and filter a small quantity of methanolic extract of *Capsicum annuum* bark. Pour this bilayer forming solution carefully down the inside of a test tube at an angle of 60°. Add 2 mL of Molisch's reagent. Violet-coloured ring appearing at interface indicates the presence of carbohydrates [44].

b. Fehling reagent (Detection of reducing sugar)

A few drops of Fehling's reagent were added to a small extract kept in a test tube, which was then heated for 30 minutes. On heating, it turned red and proved that the extract contained reducing sugars

C) Test for Glycosides

The following accompanying tests were performed using the 5 ml of methanolic leaves extracts:

a. Killer-Killani test

Add 1 mL of concentrated sulfuric acid, 1 mL of glacial acetic acid, and a trace of ferric chloride to the extract. The appearance of reddish-brown color at the junction of two layers and a blue-green color in the upper layer indicates the presence of glycosides.

b. Legal test

A few drops of 10% sodium hydroxide solution were added to the concentrated methanolic extract to make it alkaline. On adding sodium nitroprusside solution, the appearance of deep blue color confirmed the presence of glycosides.

c. Baljet test

Sodium picrate reagent was added to the methanolic extract, which produced orange and yellow colors indicating glycosides.

D) Detection of flavonoids

a. Ammonia Test

Ammonia treated filter papers, then soaked in solution with alcoholic extract. Since change of color from white to yellow on the pieces shows positive flavonoids are there.

b. Shinoda Test

A minute volume of the methanolic extract was added to a volume of ethanol and gently warmed on a few drops of the latter mixture before it was filtered off. The filtrate with magnesium flakes, along with a little concentrated hydrochloric acid gave a colour development of pink, orange, reddish purple indicating the presence of flavonoids [45-47].

E) Test for protein and amino acids

a. Millon's test

An aliquot quantity of the methanolic extract was dissolved in 5 mL of distilled water and filtered. To the contents of the filtrate taken, 1 mL of Millon's reagent was added. When a carmine-coloured precipitate formed, it assured the presence of proteins.

b. Xanthoprotein Test

Two millilitres of the extract were carefully treated with a small amount of nitric acid. The development of yellow color indicated the existence of amino acids and proteins.

c. Ninhydrin Test

One milliliter of methanolic extract was placed in a dry test tube containing two to three drops of ninhydrin reagent. It was heated in a water bath for three to five minutes and cooled to room temperature. A violet or purple coloration was thought to indicate the presence of amino acids.

d. Piotrowski's Test

Addition of 2-4 drops of a 0.02% copper sulfate solution to the ammoniated alkaline filtrate of the extract precipitated a violet color and hence proved the presence of proteins or peptides.

F) Test for Identification of Saponins

a. Foam test

A few milligrams of the residue of the sample were put into a test tube with a little distilled water. The mixture was agitated violently for nearly one minute, after which a thick lather had formed, and it remained for almost 10 minutes. Honeycomb structure foaming observation confirms the saponins existence in the extract.

G) Identification of sterols

a. Identification of Sterols by Salkowski Reaction

The extract was mixed with approximately 2 mL of concentrated sulfuric acid (H_2SO_4). Within one minute, a yellow ring that developed at the interface turned red, indicating the presence of sterols in the extract.

b. Liebermann-Burchard Reaction

A small amount of the residue (in milligrams) was placed in a test tube containing acetic anhydride and gradually heated. After cooling the test tube, a few drops of concentrated sulfuric acid were added along the side. The appearance of a blue tint in the mixture confirmed the presence of sterols.

PHARMACOGNOSTICAL INVESTIGATION

1. Fluorescence analysis

Many cut sections and crushed herbal products emit fluorescence under UV light. Identifying the latter by UV light utilizes analysis by using UV light in a number of treatments; both at 240 nm as well as 360 nm in powder medicine.

2. Determination of Ash value

One of the vital statistics for the testing of crude medicines is ash value. Ash represents non-volatile inorganic residues, which are basically ash content in any organic material.

3. Determination of Total ash

Ash content is the amount of material remaining after it has been ignited. Techniques to determine ash content are provided by the Indian Pharmacopoeia of 1996 and by the World Health Organization (WHO).

4. Determination of acid-insoluble ash

The portion that is left over after all of the ashes have been mixed with a little amount of hydrochloric acid and the remaining insoluble materials have been burnt is known as acid insoluble ash.

5. Determination of Aqueous-soluble ash

This exact ash must be boiled in 25 milliliters of water for five minutes. The insoluble matter should then be collected in Silica crucible, rinsed with warm water and ignited at a steady 450 °C.

6. Determination of Extractive value

It decides the quantity of the active principle extractible from a certain volume of drug plant

material through solvents. When crude medicine is extracted by using a particular solvent that forms a solution containing a wide range of phyto-constituents, then the extractive value of that medication indicates the quantity of polar and nonpolar chemicals included in the crude therapeutic material.

7. Determination of Foreign substance

Foreign matter present in the herbal medicines. There might be deliberate admixture or careless gathering of medicine; hence, the presence of alien particles is due to this reason.

RESULT AND DISCUSSION

Pharmacognostical Investigation

1. **Yield of methanolic extract of *Capsicum annuum* by soxhlet extraction method:** The extraction of crude drugs material was determined (Soxhlet extraction) by using 90% w/v methanol. The yields of methanolic extract were found to be 2.64 % w/w.

Table 1: Yield of *Capsicum annuum* methanolic extract in %age

Sr. No.	Quantity of powdered drug taken (kg)	Quantity of methanolic extract obtained (g)	Yield of *Capsicum annuum* methanolic extract in % (w/w)
1.	1.5	39.60	2.64

2. Preliminary phytochemical screening

Table 2: Phytochemical test of *Capsicum annuum*

Sr. No.	Major phytoconstituents	*Capsicum annuum*
1	Flavonoids	+
2	Alkaloids	+
3	Glycosides	+
4	Saponins	+
5	Proteins	+
6	Terpenoids	+
7	Carbohydrates	+
8	Resins	+
9	Phenolic compounds and tannins	+
10	Sterols	+

+ = Present, - = Absent

This table summarizes the presence of various phytoconstituents in *Capsicum annuum,* indicating that all listed components are present in the plant.

3. Ash Value

Result: The results of ash value of *Capsicum annuum are* mentioned in Tables 3.

Table 3: Determination of Ash value of *Capsicum annuum*

Sr. No	Total ash (%)	Acid insoluble ash (%)	Aq. soluble ash (%)
1.	8.2	1.9	6.3
2.	8.08	1.8	6.28
3.	8.01	1.7	6.3
AVERAGE	8.1	1.8	6.29

According to air-dried crude drug, total ash, acid insoluble ash and water-soluble ash values for *Capsicum annuum* were determined to be 8.1, 1.8, and 6.29 **%** w/w respectively.

4. Extractive value of *Capsicum annuum*

Result: The Extractive value of *Capsicum annuum* has been determined and results are given in Table 6.5

Table 4: Alcohol soluble and Water-soluble Extractive values of *Capsicum annuum*

Sr. No	(%) Alcohol soluble extracts	(%) aqueous soluble extracts
1	3.26	9.8
2	3.26	9.7
3	3.26	9.8
AVERAGE	3.26	9.8

The extractive values of *Capsicum annuum* were determined to be 3.26 percent w/w for alcohol

solubility and 9.8% w/w for water solubility, respectively.

Result: Table 5 provide the foreign matter *Capsicum annuum* results**.**

5. Foreign matter

Table 5: Foreign matter of *Capsicum annuum*

Crude Drug Wt. (gram)	After removal of foreign particle (gram) (Wt. of drugs)	Foreign matter Weight (gram)	(%) Foreign particle
10	7.65	0.15	1.18
10	7.67	0.16	1.15
10	7.66	0.17	1.17
Mean	7.66	0.16	1.16

Capsicum annuum with 1.16 percent w/w foreign matter content.

Result: The evaluation of loss on drying of *Capsicum annuum* was estimated and the observation is shown in Table 6.

6. Determination of Loss on drying

Table 6: Loss on drying in *Capsicum annuum* powder (Ten gram)

Wt. of crude herbs + Wt. of Petri plate (Before drying) (gram) A	Wt. of crude herbs + Wt. of Petri plate (After drying) (gram) B	A – B (gram)	(%) (w/w) LOD
58.2	56.150 56.138 56.148	2.15	21.5
58.1	56.560 56.342 56.320	1.7	17
58.4	56.231 56.390 56.320	2.1	21
Mean	20		

The amount of moisture in *Capsicum annuum* powder was revealed to be 20% w/w.

Result: Estimation of total phenolic content of methanolic extract of *Capsicum annuum* was provided in Table 7.

7. Total content of phenol

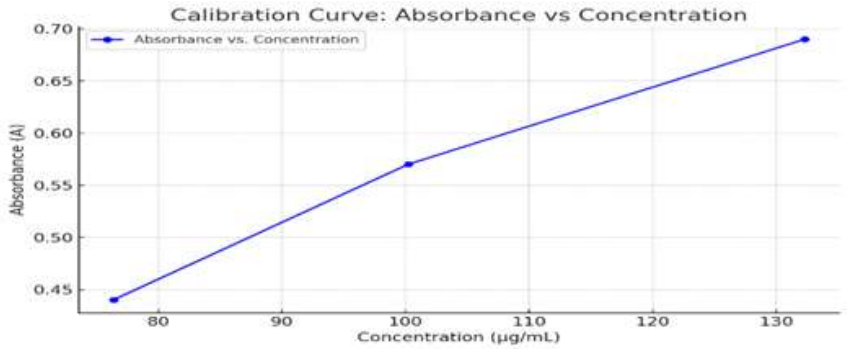

Fig. 3: Total phenolic content of calibration curve in reference to Gallic acid

Table 7: Methanolic extract's total phenol content of *Capsicum annuum*

Sr. No	Absorb. (A)	Intercept	Slope	Concentration (µg/mL)	Mean
1	0.44	0.0043	0.007	76.38	99.70 ±.20
2	0.57	0.0043	0.007	100.23	
3	0.69	0.0043	0.007	132.3	

The methanolic extract of *Capsicum annuum leaves* for total phenolic content was found to be 99.70±.020µg/mL.

8. Total flavonoid content of *Capsicum frutescent* leave extracts

Result: The methanolic extract of *Capsicum annuum* leaves of flavonoid contents has been estimated and results were given in Table 8.

Table 8: Methanolic extract's total flavonoid content of *Capsicum annuum*

Sr. No	Absorb.	Intercept	Slope	Concentration (µg/ml)	Mean
1	0.462	0.078	0.0045	97.38	97.15±1.009
2	0.538	0.077	0.0045	99.20	
3	0.626	0.078	0.0045	95.32	

The methanolic extract of *Capsicum annuum* contained 97.15±1.009 µg/mL of total flavonoids.

9. Total saponins content

Result: Total saponin content of *Capsicum annuum* extracts has been estimated and results were given in Table 9.

Table 9: The Total saponin content of *Capsicum annuum*

Sr. No.	Weight of crude herbs taken (gram)	Weight of drug obtained (gram)	Saponins content (% w/w)
1	10	0.272	2.72
2	10	0.232	2.32
3	10	0.263	2.63
Average			2.55±0.030

Capsicum annuum was concealed to have 2.55±0.030 percent w/w of total saponins.

10. Total alkaloidal content

Result: Total alkaloidal content estimated results are shown in Table 10 for the total alkaloidal content of *Capsicum annuum*

Table 10: The Total alkaloidal content of *Capsicum annuum*

Sr. No.	Crude herbs taken (gram)	Amount of crude drugs obtained (gram)	Alkaloid content (% w/w)
1	5	0.125	1.37
2	5	0.124	1.38
3	5	0.125	1.37
Mean			1.37±0.017

Adenanthera Capsicum annuum was revealed to have a total alkaloid content of 1.37 ± 0.017 percent w/w.

11. TLC profile of met. extract of *Capsicum annuum*

Table 11: TLC profile with Rf value

Types of solvent	Solvent system	Number of Spots	R_f values	visualize agent
MECA	Chloroform: methanol (8:2)	05	0.11, 0.15, 0.17, 0.20, 0.25	UV-357nm

Figure 4: TLC pictures of *Capsicum annuum* methanolic extract

CONCLUSION

In summary, the exhaustive phytochemical and pharmacological evaluation of *Capsicum annuum* has given much insight into the bioactive constituents of the plant and their potential applications in therapy. The yield of 2.64% total powder extract suggests a significant concentration of phytochemicals in the plant. The presence of important phytochemicals, such as alkaloids, flavonoids, glycosides, saponins, and terpenoids, underscores the medicinal potential of *Capsicum annuum*. The activity study of pharmacology emphasized that the extract is effective in activating Lactate Dehydrogenase, and it may play the role of a metabolizing compound. The future prospects for *Capsicum annuum* in the studies of phytochemical and pharmacology look brighter, as huge potential opens up into the exploration of further study that could substantially advance the therapeutic applications involved. The full scope for the in-depth study through advanced extraction and analytical techniques on the phytochemistry of *Capsicum annuum* should eventually come out to disclose novel bioactive compounds and their particular pharmacological effects.

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