

Pharmacological and Analytical Study of Chandrashoor Seed (*Lepidium sativum* L.) Ethanolic Extract

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Abstract

Chandrashoor (*Lepidium sativum* L.), commonly known as garden cress, is a widely cultivated plant recognized for its nutritional and medicinal properties. This study aims to explore the phytochemical composition, pharmacological activities, and analytical study of the ethanolic extract of Chandrashoor seeds using modern analytical techniques such as High-Performance Thin Layer Chromatography (HPTLC) and Fourier Transform Infrared Spectroscopy (FT-IR). The ethanolic extract was analyzed for its phytochemicals, including alkaloids, flavonoids, phenolic compounds, and glucosinolates. The pharmacological evaluation revealed significant antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. The therapeutic potential of the extract was further validated by its ability to manage oxidative stress, inflammation, and microbial infections. The study also emphasizes the importance of utilizing advanced techniques such as HPTLC and FT-IR for detailed analysis of complex plant extracts.

Key words : *Lepidium sativum* L., *Chandrashoor*, ethanolic extract, HPTLC, FT-IR, phytochemical analysis, pharmacological activities, analytical study.

Chandrashoor (*Lepidium sativum* L.) has been widely used in traditional medicine for its various health benefits, including its use as an antioxidant, anti-inflammatory, and antimicrobial agent. The seeds of this plant are rich in bioactive compounds such as flavonoids, alkaloids, glucosinolates, and phenolic acids. Despite the extensive use

of Chandrashoor in folk medicine, there is limited scientific data on its comprehensive phytochemical profile and pharmacological activities⁹. High-Performance Thin Layer Chromatography (HPTLC) and Fourier Transform Infrared Spectroscopy (FT-IR) are advanced analytical techniques that provide detailed information on the chemical composition

and molecular structure of plant extracts. This study aims to evaluate the phytochemical composition of Chandrashoor seed ethanolic extract using these techniques and investigate its pharmacological activities.

Morphology :

Garden cress (*Lepidium sativum* L.) seeds are smooth, small and reddish brown in color. Shape is oval with point and triangular at one end. Seed length is about 3-4 mm and wideness is 1 -2 mm. When seed is soaked in water seed coat swells and gets covered with transparent, colorless, mucilage and give mucilaginous taste^{3,13}.

Microscopic characters :

The microscopic features of **Garden cress** (*Lepidium sativum* L.) seeds reveal key details about their structure:

Endosperm :

- The **endosperm** consists of **thick-walled polygonal cells**, indicating a strong, geometrically shaped cellular structure that serves as a food reserve for the developing seed.

Embryo :

- The **embryo** is made up of **small polygonal cells**, which are minute in size compared to the endosperm. These cells are encased by the endosperm, providing protection and nutrients for growth.

Seed powder :

- When ground into powder, the **seed**

powder has a **creamish yellow hue**. It shows a consistent structure with **uniform thick-walled cells** and contains **reddish-brown fragments of the seed coat**, which may indicate the presence of pigments.

- The **endosperm** in the powder is oily, suggesting a rich source of fats.
- The presence of **reddish coloring matter** may be attributed to natural pigments like flavonoids or anthocyanins^{1,3,12}.

Chemical constituents :

The quantitative analysis of *Lepidium sativum* seeds showed that the seeds contained protein (24.2±0.5%), lipids (23.2±0.2%), carbohydrates (30.7±1.2%), fiber (11.9±0.4%), ash (7.1±0.1%), moisture (2.9±0.1%), alkaloids (0.40%), flavonoid 0.42%), saponin (2.8%), tannin (0.61%) and phenol (0.004%)^{6,10}.

Component	Content (%)
Protein	24.2 ± 0.5
Lipids	23.2 ± 0.2
Carbohydrates	30.7 ± 1.2
Fiber	11.9 ± 0.4
Ash	7.1 ± 0.1
Moisture	2.9 ± 0.1
Alkaloids	0.40
Flavonoids	0.42
Saponins	2.8
Tannins	0.61
Phenols	0.004

Analysis of methanolic seeds extract of *Lepidium sativum*, showed that the extract contained 46 compounds included deoxyspergualin, 6-oxabicyclo [3.1.0] hexan-3-one,

2-furancarboxaldehyde, 5-methyl, 9-Oxabicyclo [3.3.1] nonane-2,6-diol, glycyL-dL-serine, 2-hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin, methyl nicotinate, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, thiocyanic acid, octyl ester, maltose, benzofuran, 2,3-dihydro, 5-hydroxymethylfurfural, 2-methoxy-4-vinylphenol, ascaridole epoxide, phenol, 2-methoxy-5-(1-propenyl)-, (E), α -D-glucopyranoside, O- α -D-glucopyranosyl-(1-fwdarw)- β -D-fruc, 2h-Indeno[1,2-b]furan-2-one, 3,3a, 4,5,6,7,8,8b-octahydro-8,8-dim, limonen-6-ol, pivalate, (5 β) Pregnane-3, 20 β -diol, 14 α , 18 α -[4-methyl3-oxo-(1-oxa-4-azabul, cinnamic acid, 4-hydroxy-3-methoxy-, {5-hydroxy-2-hydroxymethyl, 9-oximino-2,7-diethoxy fluorene, Phorbol, Streptovitamin A, 4,25-secoobscurinervan-4-ol,6,7-didehydro-22-ethyl15,16-dimethy, desulphosinigrin, d-mannose, methyl (1-O-retinyl2,3,4-triacetyl- β -D-glucopyran) urinate, tetraacetyl-d-xylonic nitrile, dasycarpidan-1-methanol, acetate (ester), octadecanoic acid, 1Hcyclopropa [3,4] benz[1,2-e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a, oxiraneoctanoic acid 3-octyl-,cis, 9-octadecanamide, (Z), octadecanal, 2-bromo, tributyl acetylcitrate, pyrrolidine, 1-(1-oxo-7,10-hexadecadienyl), 8H-Azecino[5,4-b]indol-8-one, 5-ethylidene- 1,2,3,4,5,6,7,9-octahy, 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one, 2H-benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one, 9-[2-(dimethylan, pregn-5-ene-3,8,11, 12,14,20-hexol, (3 β ,11 α ,12 β ,14 β ,20R), α -tocopherol, vitamin E, 6,7-epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate, stigmasterol, 9,19-cyclolanostane-3,7-diol and ergosta-5,22,-dien-3-ol,acetate, (3 β ,22E)⁷.

Lepidium sativum ethanolic extract contained total phenolics 4.46 \pm 0.14 to 11.03 \pm 0.75 (mg GAE/g dw plant material) and

flavonoids of 3.57 \pm 1.2 to 4.79 \pm 0.24 (mg QE/ 100 g dw plant material). Phenolics identified in the ethanolic extract of *Lepidium sativum* were kaempferol, coumaroylquinic acid, p-coumaroyl glycolic acid and caffeic acid. The isoflavonoids: 5,6-dimethoxy-2',3'-methylenedioxy-7-C- β -d-gluco-pyranosyl isoflavone, 7-hydroxy-4',5,6-trimethoxy isoflavone and 7-hydroxy-5,6-dimethoxy-2',3'-methylenedioxyisoflavone were isolated from *Lepidium sativum*.¹²

Plant material collection :

The seeds of *Lepidium sativum* were collected from a local herbarium and identified by a botanist. The seeds were dried under shade and powdered for the preparation of the ethanolic extract.

Preparation of Ethanolic extract :

The powdered seeds (50 g) were subjected to solvent extraction using ethanol (80%) in a Soxhlet apparatus for 48 hours. The extract was concentrated using a rotary evaporator and stored in a cool, dry place for further analysis.¹¹

Phytochemical screening :

Standard methods were employed to screen the ethanolic extract for the presence of major phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, tannins, and glucosinolates. Qualitative tests were performed based on the formation of color reactions and precipitates.

HPTLC analysis :

The ethanolic extract was analyzed by

High-Performance Thin Layer Chromatography (HPTLC) using silica gel plates as the stationary phase. The mobile phase consisted of chloroform: methanol (9:1). The developed plates were visualized under UV light at 254 nm and 366 nm. The chemical profile was documented and compared with standard markers.

Instrumentation and conditions :

The HPTLC analysis was performed using a CAMAG HPTLC system equipped with a Linomat V sample applicator, TLC Scanner 3, and a CATS 4 software. Pre-coated silica gel 60 F254 plates (20 cm × 10 cm) were used for the analysis. The mobile phase consisted of a mixture of toluene, ethyl acetate, and formic acid in the ratio of 6:4:0.5 (v/v/v). The chamber was pre-saturated for 30 minutes to achieve a consistent environment. Samples (5 µL) of the ethanolic extract and standards were applied as bands on the plate at a constant application rate.

Detection and Visualization :

After separation, the chromatograms were developed and visualized under UV light at wavelengths of 254 nm and 366 nm. Additionally, derivatization using anisaldehyde-sulfuric acid reagent was performed to enhance the visibility of the phytochemical compounds. The plates were then heated at 105°C for 5 minutes to develop the color reaction.

Standardization and comparison :

To identify specific phytochemicals, standard reference compounds, such as quercetin (flavonoid), caffeine (alkaloid), and β-sitosterol

(sterol), were used. The R_f values (retention factor) of the spots from the seed extract were compared with those of the standards.

Quantification :

Densitometric analysis was performed using the TLC scanner to quantify the compounds based on their peak areas. A calibration curve was generated using different concentrations of the reference standard compounds.

FT-IR Analysis :

Fourier Transform Infrared Spectroscopy (FT-IR) was used to identify the functional groups present in the ethanolic extract. The sample was prepared as a thin film on the KBr pellet and analyzed using an FT-IR spectrophotometer in the range of 4000–400 cm⁻¹.

FT-IR is a technique used to identify the functional groups of compounds based on their molecular vibrations. It provides a spectrum that represents the absorption of infrared light at different wavelengths corresponding to various functional groups in the compounds.

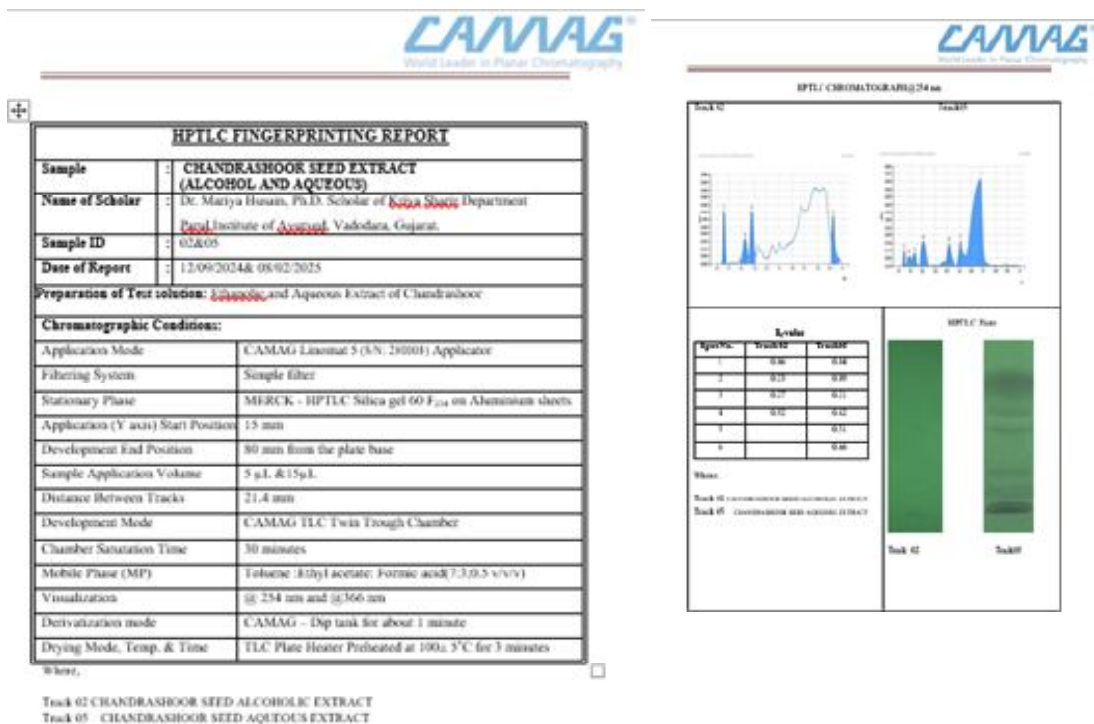
- **Sample Preparation for FT-IR :** The ethanolic extract of *Lepidium sativum* seeds is first dried and then ground with potassium bromide (KBr) to form a pellet or thin film for FT-IR analysis.
- **FT-IR Analysis :** The FT-IR spectrum is obtained by passing infrared light through the sample and measuring the absorbance at different wavelengths. Functional groups can be identified by their characteristic absorption bands.

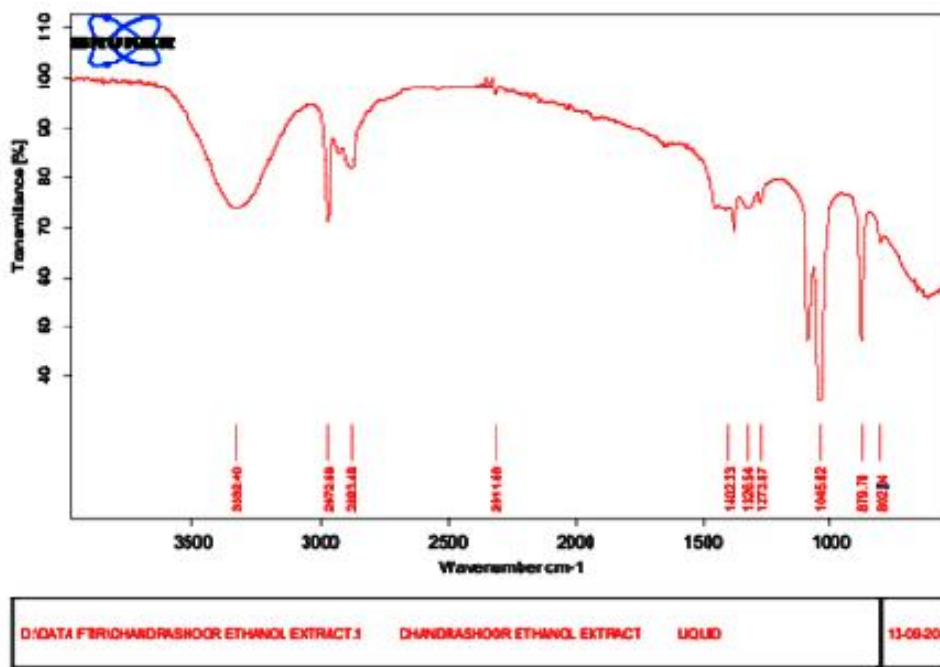
- **Key Functional Groups :**
 - **Hydroxyl groups (–OH):** Broad absorption bands around 3200–3600 cm^{-1} .
 - **Carbonyl groups (C=O):** Strong absorption around 1700 cm^{-1} .
 - **Aromatic C–H stretching :** Absorptions around 3000 cm^{-1} .
 - **C–O stretching:** Absorption around 1200–1300 cm^{-1} .
- **Identification of compounds :** The FT-IR spectrum of the extract will display characteristic peaks corresponding to functional groups associated with bioactive compounds such as flavonoids, alkaloids, glucosinolates, and phenolic compounds.

Pharmacological properties of Chandrashoor seed Ethanolic extract :

Pharmacological studies of *Lepidium sativum* seeds have demonstrated several potential therapeutic effects, such as:

Diuretic activity - The diuretic effect of aqueous and methanolic extracts of the Garden cress (*Lepidium sativum*) seeds in adult male Wistar rats. Extracts were administered orally to experimental rats at doses of 50 and 100mg/kg body weight. Both the extracts of Garden cress (*Lepidium sativum*) seeds showed a dose-dependent increase in urine excretion. The excretion of sodium was increased by both the extracts and potassium excretion was increased only by the aqueous extract at a dose of 100 mg/kg. The





methanolic extract had the additional advantage of a potassium-conserving effect. Aqueous and methanolic extracts of seeds showed notable diuretic effect which is comparable to that produced by the reference diuretic hydrochlorothiazide.⁹

Effect on sperm parameter - In study conducted by Naji⁹ the effect of phenol extract of Garden cress (*Lepidium sativum*) seeds on sperm parameters of adult male rabbits. Their result shows that Medium Effect Dose (MED50) of phenols was obtained by Dose-Response Curve. MED50 of Garden cress (*Lepidium sativum*) seeds seed phenols was 36.1 mg/kg body weight. There was a significant increase in testicular sperm concentration, epididymus sperm concentration and in the sperm count per gm of the testis, sperm motility percent, grade activity, sperm

viability percent, and abnormal sperm morphology percent of epididymis caudal at MED50 of Garden cress (*Lepidium sativum*) seed phenol. Authors reported that supplementation with low doses of *Lepidium sativum* seed phenols could enhance rabbit fertility. Researcher concluded that phenol extract of *Lepidium sativum* seeds improves some parameters of sperm¹⁴.

Hepatoprotective effect - Researcher examined hepatoprotective effect of Garden cress (*Lepidium sativum*) seed methanolic extract for the prevention of carbon tetrachloride (CCl₄) induced liver damage. Garden cress (*Lepidium sativum*) seed methanolic extract (200 and 400 mg/kg body weight) was administered to rats having induced liver injury. Serum activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine

aminotransferase (ALT) and bilirubin concentration were increased significantly in the group of rats received only CCl₄. There was a significant reduction in these parameters in groups administered with Garden cress (*Lepidium sativum*) seed extract, the severe fatty changes in the livers of rats caused by CCl₄ were also decreased. The researcher results shows that the methanolic extract of Garden cress (*Lepidium sativum*) seeds seems to possess hepatoprotective activity in rats.⁵

Antimicrobial activity : Petroleum ether extract of seed in concentrations of 2.5%, 5% and 10% had active antimicrobial effect against six different pathogens together with powerful antifungal activity at the concentration of 2.5 and 10% 35. Ethanolic extract of seed was found very effective against fungal growth (*Fusarium equisetia*, *Aspergillus flavus* and *Alternaria alternata*) at 2-8% of seed extracts.²

Toxicity and safety :

Datta *et al.*⁴ conducted a study on adult rats to assess the safety of Garden cress seed. For the acute toxicity study, rats were fed seed powder 0.5 – 5.0 g/kg body weight mixed with standard diet and symptoms of toxicity and mortality were monitored for 72 hours. No toxicity and mortality symptoms were found in rats.⁵ The absence of any toxicity symptoms or mortality indicates that Garden Cress seeds are safe for consumption within this dose range. This study supports the potential use of Garden Cress as a safe natural remedy or food supplement.

1. *Phytochemical Profile and Fingerprint (HPTLC)*:

- The HPTLC analysis of the ethanolic extract reveals several prominent bands corresponding to different classes of bioactive compounds. Common compounds identified include flavonoids (*e.g.*, quercetin), alkaloids, glucosinolates, and phenolic acids.
- The R_f values of these bands help in identifying and quantifying the individual compounds. For example, quercetin may show a characteristic R_f value in the extract.

FT-IR Spectral Analysis :

- The FT-IR spectrum of the ethanolic extract displays several key functional group absorptions. For instance, a broad absorption band around 3400 cm⁻¹ suggests the presence of hydroxyl groups (–OH), indicative of phenolic compounds or flavonoids.
- A strong absorption near 1700 cm⁻¹ confirms the presence of carbonyl (C=O) groups, which are characteristic of flavonoid aglycones or other aromatic compounds.
- The presence of C–O stretching bands around 1200–1300 cm⁻¹ further suggests the existence of glycosides or other esterified compounds.

Pharmacological Correlation :

- The identified compounds from HPTLC and FT-IR correlate with the known pharmacological properties of *Lepidium sativum*. For example, flavonoids and phenolic compounds (identified via

HPTLC and FT-IR) are known to contribute to antioxidant and anti-inflammatory activities.

- Alkaloids and glucosinolates are likely responsible for the antimicrobial and potential anticancer effects observed in the extract.

Standardization and Quality control :

- The HPTLC fingerprint and FT-IR spectrum serve as essential tools for the standardization of the ethanolic extract. The chromatogram provides a unique identification pattern that can be used for batch-to-batch consistency, ensuring the extract's quality and potency.
- *Discussion on Diuretic activity* - Both the aqueous and methanolic extracts of Garden Cress seeds showed a dose-dependent increase in urine output, which is a key indication of diuretic activity. The methanolic extract had the additional benefit of conserving potassium, unlike the aqueous extract at 100 mg/kg. This feature makes the methanolic extract a safer option in terms of reducing the risk of hypokalemia, a condition that can occur when excessive potassium is lost during diuresis. This suggests that Garden Cress extracts could be considered a natural alternative with diuretic potential.
- *Discussion on Effect on Sperm Parameter*- The phenolic extract from Garden Cress (*Lepidium sativum*) seeds significantly improved several sperm parameters in male rabbits. The Medium Effect Dose (MED50) was determined to be 36.1 mg/kg body weight. At this dose, there was a notable increase in testicular and epididymal sperm concentration, sperm motility, viability, and sperm morphology, alongside a reduction in abnormal sperm. These results suggest that Garden Cress seed phenols have a positive effect on sperm quality and fertility. The antioxidant properties of phenolic compounds likely contribute to the observed improvements in sperm health. This research indicates the potential of Garden Cress as a natural fertility enhancer, warranting further studies for possible applications in human fertility.
- *Discussion on hepatoprotective* - The study showed that Garden Cress (*Lepidium sativum*) seed methanolic extract has hepatoprotective effects in rats with carbon tetrachloride (CCl₄)-induced liver damage. It significantly reduced elevated liver enzymes (ALP, AST, ALT) and bilirubin, and decreased fatty changes in the liver. These findings suggest that Garden Cress seed methanolic extract possesses hepatoprotective properties, likely due to its antioxidant and anti-inflammatory effects, which could be valuable in preventing or treating liver damage.
- *Discussion on Antimicrobial property* - The petroleum ether extract showed effective antimicrobial activity against six different pathogens, with notable antifungal effects at concentrations of 2.5% and 10%. The ethanolic extract was particularly effective against fungal growth, inhibiting *Fusarium equiseti*, *Aspergillus flavus*, and *Alternaria alternata* at concentrations ranging from 2% to 8%. These findings suggest that Garden Cress seed extracts possess significant antimicrobial and antifungal properties, which could be harnessed for therapeutic applications or in natural preservative formulations.

The ethanolic extract of *Lepidium*

sativum seeds demonstrates promising pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and antidiuretic effects. HPTLC analysis offers a detailed profile of the bioactive compounds, while FT-IR spectroscopy provides information on the functional groups present, aiding in the identification and characterization of these compounds.

The combination of HPTLC and FT-IR offers an effective approach for the comprehensive analysis and standardization of the extract, supporting its potential therapeutic applications. These techniques also ensure quality control, making them valuable tools in the development of herbal formulations. Garden Cress (*Lepidium sativum*) seeds exhibit a range of beneficial biological activities. The aqueous and methanolic extracts demonstrate significant diuretic effects, with the methanolic extract offering the added advantage of potassium conservation, making it a safer diuretic option. Additionally, the phenolic extract improves sperm quality and fertility in male rabbits, highlighting its potential as a natural fertility enhancer. The methanolic extract also shows hepatoprotective properties by reducing liver enzyme levels and fatty liver changes in rats, suggesting its potential for liver health support. Lastly, both the petroleum ether and ethanolic extracts exhibit strong antimicrobial and antifungal activities, indicating their usefulness in therapeutic and preservative applications. These findings indicate that Garden Cress seeds have the potential for a variety of health benefits and should be explored further for broader applications. Further research could explore the exact mechanisms behind the pharmacological effects of *Lepidium sativum* and its bioactive

compounds, as well as clinical trials to establish its efficacy in humans.

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Conflict of interest

The authors declare no conflict of interest.

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