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Pharmacognostic Characterization, Phytochemical Profiling, and High-Performance Thin-Layer Chromatographic (HPTLC) Fingerprinting of *Cannabis sativa* L. Seeds and Leaves

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ABSTRACT

Introduction: *Cannabis sativa* L. (*C. sativa*) is a medicinally and nutritionally important plant, traditionally used for its therapeutic benefits. Even with the increasing interest in its applications, standardized pharmacognostic data on its seeds and leaves remain limited. This study was proposed to establish a comprehensive phytopharmacognostic profile of C. sativa leaves and seeds to maintain its safe and effective use in herbal formulations and dietary products.
Methods: Macro and microscopic evaluations were conducted to identify diagnostic botanical characters of the plant samples. Preliminary phytochemical screening was performed on methanolic extracts of both leaves and seeds to identify key bioactive compounds. Physicochemical parameters, including moisture content, total ash, and extractive values, were assessed in accordance with WHO/API guidelines. HPTLC profiling of the samples was performed to identify the quality and for safety evaluations. Heavy metal analysis (Pb, Cd, As, Hg), aflatoxin content, pesticide residues, and microbial load were carried out according to the standards of the Ayurvedic Pharmacopeia of India (API).
Results: Microscopic analysis revealed glandular and simple trichomes in leaves, and aleurone grains, oil globules, and parenchyma cells in seeds. Phytochemical screening showed the presence of alkaloids, tannins, flavonoids, phenols, and saponins in both samples. Glycosides were found only in leaves, while proteins were exclusive to seeds. All physicochemical parameters were within acceptable pharmacopoeial limits. HPTLC profiling of both extracts shows the presence of various spots, in which 0.606 & 0.501 in the leaf and 0.584 & 0.480 in the seed resemble lupeol and stigmasterol, respectively. Heavy metals, aflatoxins, and pesticide residues were all below permissible limits, and microbial counts met safety standards, indicating that the plant materials were of high quality and uncontaminated.

Conclusion: The study demonstrates the distinctive pharmacognostic markers and phytochemical framework of *C. sativa* leaves and seeds. The absence of significant contaminants and compliance with API and WHO quality standards affirm the plant's suitability for inclusion in standardized herbal and nutraceutical formulations. These findings contribute valuable reference data for regulatory, pharmaceutical, and research purposes.

INTRODUCTION:

Cannabis sativa L. (*C. sativa*), an herbaceous annual plant belonging to the Cannabinaceae family. The plant mainly originates from Central and Eastern Asia, and is now cultivated worldwide, thriving in moderate to subtropical regions with full sun and well-drained soils.[1] Although its legal status varies throughout the world, it is used for industrial, medical, and recreational uses.[2] It grows wild in India from Kashmir to Assam and in tropical lowlands, but unlicensed cultivation is prohibited under the Narcotic Drugs and Psychotropic Substances Act, 1985. Though leaves and seeds without flowering tops may be legally used for industrial or medicinal purposes.[3]

For millennia, several cultures have used the plant for ethno-medicines, nutritional, industrial, and, owing to its therapeutic potential and psychotropic properties. An in-depth investigation of its ancient, biochemical, and therapeutic dimensions provides the context for its contemporary understanding. The plant embraces a diverse range of phytochemicals, including cannabinoids, alkaloids, flavonoids, phenolic compounds, tannins, and essential fatty acids [4], that contribute to a vast range of physiological activities, including anti-inflammatory, antioxidant, analgesic, and neuroprotective effects, etc.[5] Among its diverse components, the seeds and leaves are especially fascinating. The seeds are high in protein and polyunsaturated fatty acids[6], while the leaves contain a number of secondary metabolites, including cannabinoids, such as tetrahydrocannabinol (THC), and cannabidiol, etc., with potential therapeutic uses[7].

Pharmacognostic assessment, which includes macroscopic and microscopic evaluation, and physicochemical examination, is required for the accurate identification, authenticity, and quality control of medicinal plant materials.[8] These estimations are crucial for establishing pharmacopoeial standards and ensuring batch-to-batch consistency, particularly in the development of herbal formulations. Phytochemical screening contributes to this by identifying the various bioactive compounds in the plant sample responsible for its therapeutic effects and supporting the preliminary assessment of its pharmacological potential.

C. sativa is known as "*Bhanga*" in Ayurvedic literature and is categorized under *Upavisha* (sub-toxic drug) *Varga*, signifying its fast action or high-level bioactivity. Classical literature highlights the medicinal value of *Bhanga* when purified appropriately, which supports the current concept that thorough processing and standardization are crucial for the safe use of Ayurvedic formulations or drugs. The prolonged use of the plant also leads to a range of negative physical and mental health effects, including impaired cognitive function, anxiety, paranoia, etc.[9]

The present study aims to provide a comprehensive pharmacognostic and phytochemical exploration of the seeds and leaves of *C. sativa*. The data is expected to support the quality standardization, scientific validation, and facilitate its accountable use in modern and Ayurvedic formulations.

Materials and Methods

Materials and reagents

All chemicals, reagents, and solvents used in the experiments were of analytical grade, and all research activities were conducted at CARI Jhansi, Uttar Pradesh.

Plant materials collection and authentication

C. sativa Linn. leaves and seeds were procured from Gem Herbal GMP Certified Pharmaceuticals Company and authenticated by the Department of Pharmacognosy, Central Ayurveda Research Institute, Jhansi. The authentication specimen was deposited in the Department of Pharmacognosy of the institute and is available for reference. (Figure 1)

Figure 1: Collection and authentication of the plant

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Figure 1.1: Fresh plant of *C. sativa* L.

Pharmacognostical studies:

The macroscopic parameters, viz., texture, shape, size, color, etc., of the leaf and seed of *C. sativa* L. were observed by naked eye and with a simple microscope. A free-hand section of the plant samples was taken with a clean, sharp diamond-edge blade, and the section was mounted on slides in 50% glycerine and observed under a binocular compound microscope. The sections of the sample were observed to examine their cell contents, and then stained with other reagents.[10]

Physicochemical evaluation

The physicochemical parameters, including loss on drying, extractive values, ash values, pH value, and other heavy metals, pesticide residues, and aflatoxin tests, of the leaves and seeds of C. sativa L. samples were determined according to the Ayurvedic Pharmacopeia of India, Part I, Vol. I.[11]

HPTLC profiling

The extract (3 µL) of leaves and seeds of *C. sativa* L. was applied in the form of 8 mm bands, 15 mm from the bottom of a 10 cm × 10 cm preactivated aluminum-supported precoated silica gel 60F254 plate, with the help of an ATS-4 applicator attached to a CAMAG HPTLC system. The plate was developed using stationary phase TLC silica gel 60 F 254 on an aluminum plate saturated twin trough chamber using the mobile phase as Ethyl acetate: Formic acid (7.0: 3.0: 0.2 v/v/v) to a distance of 8 cm, dried for 5 minutes at room temperature. The chromatogram was then documented by capturing images under ultraviolet (UV) illumination at 254 nm and 366 nm wavelengths, as well as in daylight conditions, to visualize the separated constituents.[12]

Observation and Result:

Macroscopic characters

Organoleptic characters of the plant, initially identified by its appearance, color, odor, shape, dimensions, etc., are shown in Table 1:

Table 1: Organoleptic characters of *C. sativa* leaves and Seeds

Sr. no.	Characters	Leaves (dry)	Seeds
1.	**Color (*Roop*)**	Brownish Green	Brownish Green,
2.	**Taste (*Ras*)**	Bitter (*Tikta*)	Mild Nutty Flavor
3.	**Odor (*Gandha*)**	Specific	Specific
4.	**Touch (*Sparsh*)**	Rough	Sub-spherical in shape
5.	**Dimensions**	4–7 cm X 0.2–1 cm	1.54–2.61 mm X 1.38–1.94 mm

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Microscopic characters of *C. sativa* fresh leaves and seeds

Transfer section of leaf:

The transverse section of the midrib and lamina of *C. sativa* leaf showed the presence of the upper and lower epidermis surfaces. The epidermis was undulating, with the presence of glandular and non-glandular trichrome. The midrib comprised collenchyma's layer cells underneath the upper and lower epidermis, and parenchyma containing the aggregate crystal. The mesophyll showed of palisade layer and parenchyma cells containing aggregated crystals. Sclerenchyma tissue was observed surrounding vascular bundles. (Figure 2)

Figure 2: Transfer section of leaf through the midrib

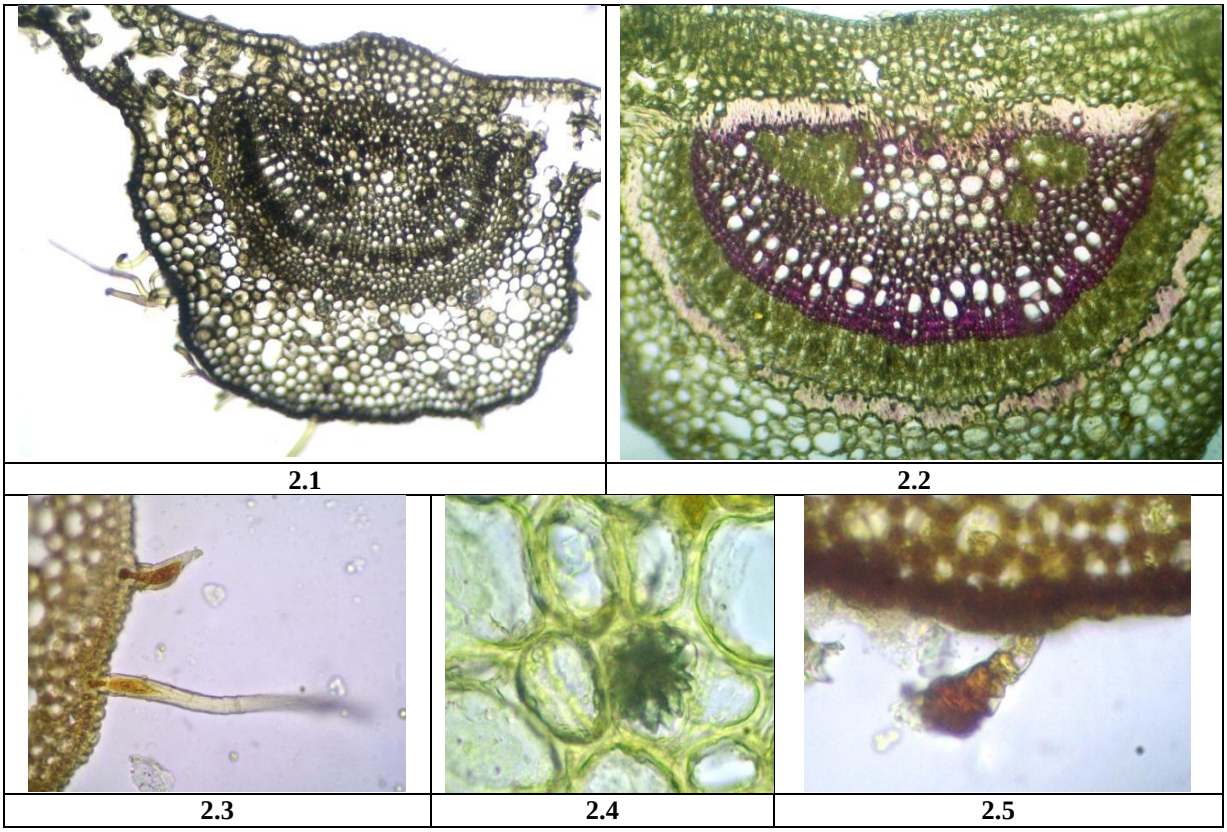
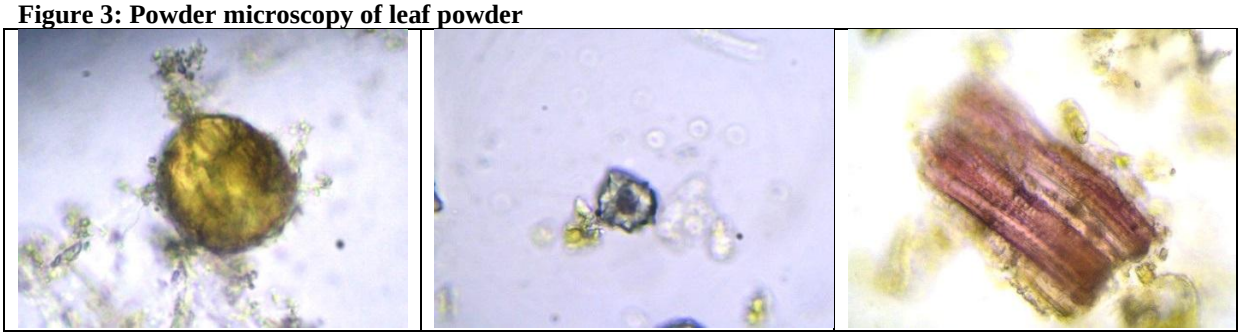


Figure 2: 2.1: T.S. through Midrib with Central Vascular Bundle, **2.2:** Central Vascular bundle with Xylem & Phloem, **2.3** Epidermis with Trichome, **2.4** Parenchyma Cells with Cluster Crystals, **2.5** Glandular Trichomes

Powder microscopy of leaf powder

On powder microscopy, the presence of unicellular trichrome, Simple fiber, brown content, oleoresin content, palisade, parenchyma, aggregate crystals, and spiral vessels. (Figure 3)

Figure 3: Powder microscopy of leaf powder



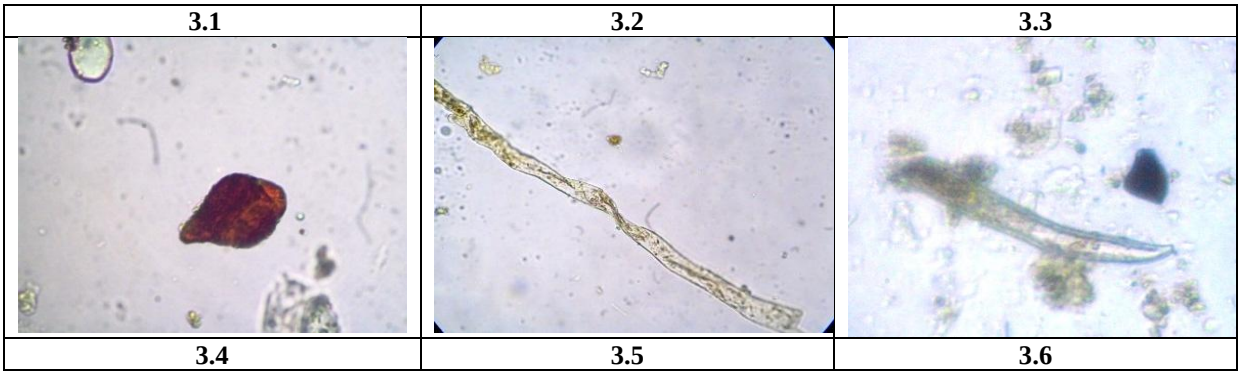
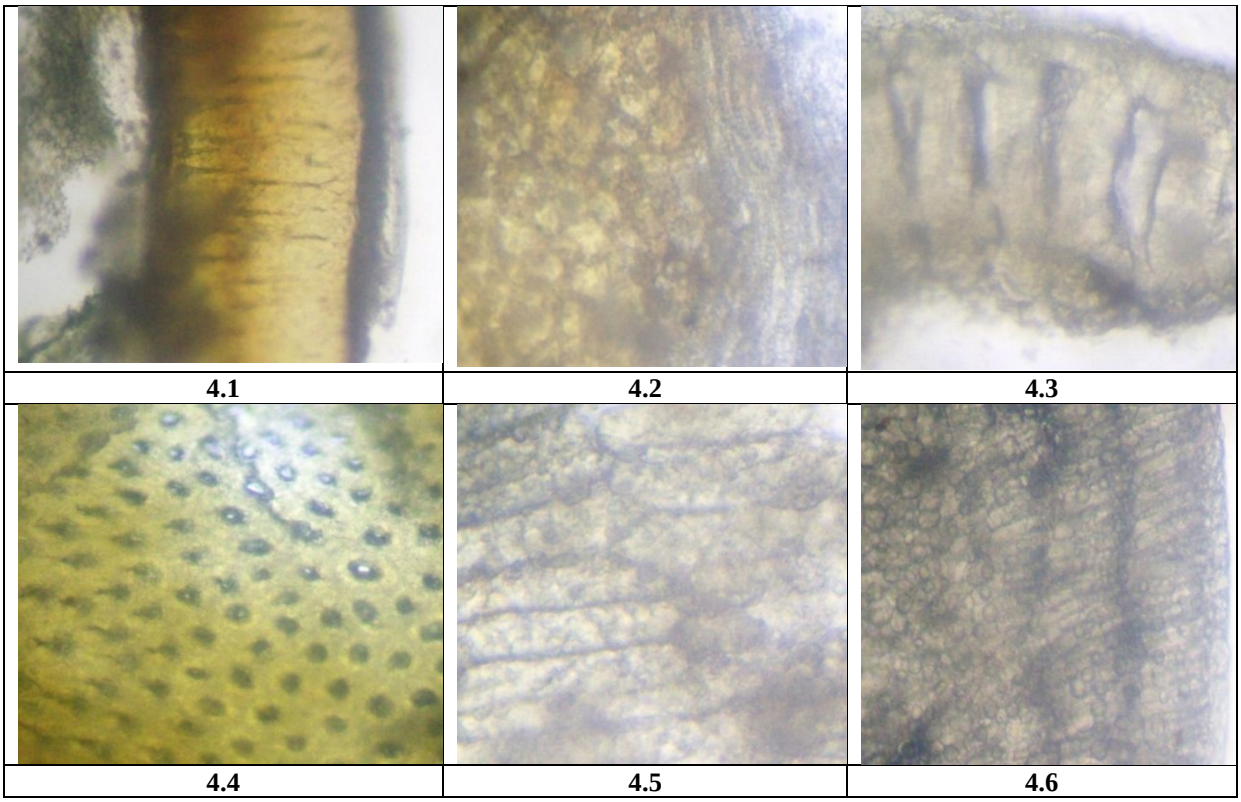


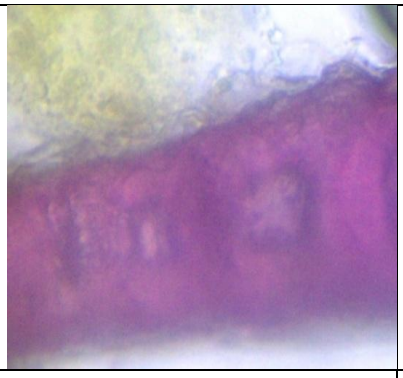
Figure 3: 3.1: Oleoresins Content, **3.2:** Crystal, **3.3:** Spiral Vessels, **3.4:** Brown content, **3.5:** Fibres, **3.6:** Trichomes

Transfer section of seed:

The outer testa is very hard, composed of epidermal cells followed by a palisade cell layer. A stone cell layer is present around the kernel in a circular arrangement. The kernel consists of thin-walled parenchyma cells with oil globules, cluster crystals, and aleurone grains. Some of the vascular strands are also observed, followed by a layer of small rectangular cells that leads into to endosperm. Endosperm consists of tangentially running parenchyma cells embedded with oil globules, starch grains, and cluster crystals of Calcium oxalate. The cotyledon upper and lower epidermis is composed of squarish-shaped cells with 8-9 layers. (Figure 4)

Figure 4: Transfer section of seed shows outermost testa, tegma & inner endosperm





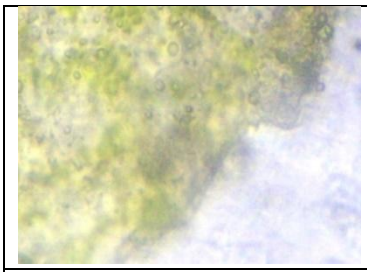
4.7

Figure 4: 4.1: Outermost Testa with Palisade cells, **4.2:** Parenchyma cells with oil globules, **4.3:** Stone cell layer, **4.4:** Epidermal cells, **4.5:** Pallisade cells, **4.6:** Part of endosperm, **4.7:** Lignified stone cells

Powder microscopy of seed powder

The diagnostic characters of powder show the presence of oil globules, parenchyma cells, spool cells, aleurone grains, fibers, and epidermal cells. (Figure 5)

Figure 5: Powder microscopy of seed powder



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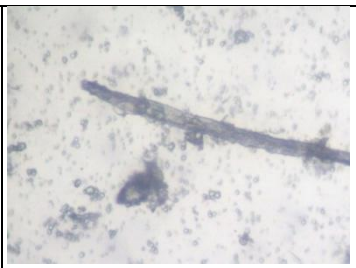

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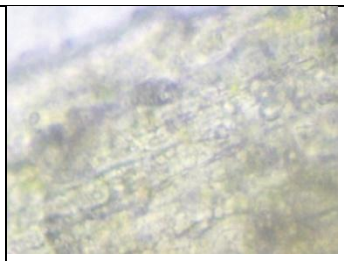

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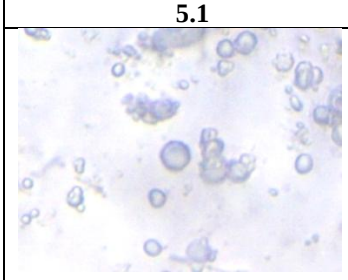

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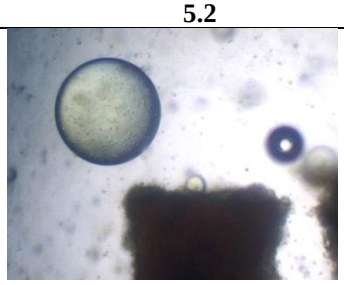

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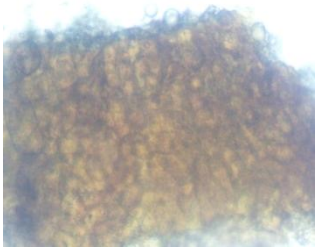

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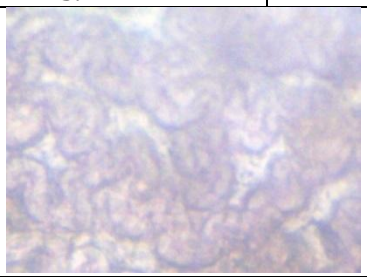

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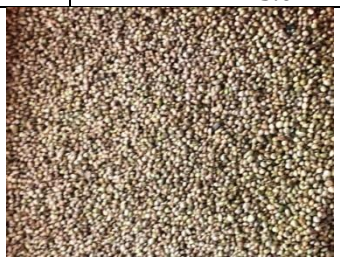


5.8

Figure 5: 5.1: Epidermal cells, **5.2:** Fibers, **5.3:** Palisade cells, **5.4:** Aluerone grains, **5.5:** Oil globules, **5.6:** Epidermal cells, **5.7:** Endosperm cells, **5.8:** *C. sativa* seeds

Analytical study of *C. sativa* Leaves and Seeds

The Phytochemical Analysis of *C. sativa* Leaves and Seeds is shown in Table 2.

Table 2: Phytochemical Analysis of *C. sativa* Leaves and Seeds;

Sr.no	Functional Group	Tests	Observation	Leaves	Seeds
1	**Glycosides**	Keller-kiliani test	Reddish brown color turns to bluish green	+	+
2	**Alkaloids**	Dragendroff's test	Orange + red color	+	+
		Mayer's test	Creamy color	+	++
		Wagner's test	Brownish color	+	+
3	**Tannins & Phenols**	Ferric chloride test	Greenish black color	+	+
4	**Proteins**	Xanthoproteic test	Dark yellow color	-	+
5	**Flavonoids**	Shinoda test	Orange or deep red color	+	+
		Lead acetate test	Yellow color	+	-
6	**Saponins**	Salkowski test	Foam present	+	+

+ve for Present, -ve for absent

Physicochemical evaluation

The results of physicochemical parameters, heavy metal analysis, Pesticide Residue Analysis, and Aflatoxin analysis are presented in Tables 3, 4, 5, and 6, respectively, for both leaves and seeds of *C. sativa*.

Table 3: Physicochemical evaluation of *C. sativa* Leaves and Seeds;

Sr.no.	Parameter	Leaves	Limit range	Seeds	Limit range	Reference
1	Foreign matters (%)	ND	NMT 2%	ND	NS	API Part I Vol. I
2	Loss on drying	7.60±0.05	NS	6.72±0.01	NS	
3	pH (10% aq Sol.)	8.71±0.02	NS	6.22±0.02	NS	
4	Water-soluble extractive value (%w/w)	21.25±0.30	NLT 13 %	8.40±0.25	NS	
5	Alcohol-soluble extractive value (%w/w)	11.73±0.04	NLT 10%	13.45±0.03	NS	
6	Total ash (%w/w)	14.97±0.01	NMT 15%	5.80±0.02	NS	
7	Acid insoluble-ash (%w/w)	4.59±0.03	NMT 5%	1.63±0.02	NS	

***ND:** Not detected, **NS:** Not specified

Table 4: Test for Heavy Metals

S. No.	Parameter	Result (In seed and leaf sample)	Permissible Limit	Method Used	Reference
1	Lead (Pb)	Below the Detection limit	NMT 10 ppm	USP<233>	API Part-I, Vol-II
2	Cadmium (Cd)		NMT 0.3 ppm		
3	Arsenic (As)		NMT 3 ppm		
4	Mercury (Hg)		NMT 1 ppm		

Table 5. Permissible Limits of Pesticide Residues

S.	Pesticide Residue	Result	Permissible	Method	Reference

No.		(Seed and Leaf sample)	limit (mg/kg)	Used	
1.	Alachlor	Below the Detection limit	0.02	AOAC official methods 2007.01	API Part-I, Vol-II
2	Aldrin and Dieldrin (sum of)		0.05		
3	Azinphos-methyl		1.0		
4.	Bromopropy late		3.0		
5	Chlordane (sum of cis-, trans - and Oxythlordane)		0.05		
6	Chlorfenvinphos		0.5		
7	Chlorpyrifos		0.2		
8	Chlorpyrifos-methyl		0.1		
9	Cypermethrin (and isomers)		1.0		
10	DDT (sum of p.p'-DDT, o,p'-DDT, p,p-DDE and p,p'-TDE)		1.0		
11	Deltamethrin		0.5		
12	Diazinon		0.5		
13	Dichlorvos		1.0		
14	Dithiocarbamates (as CS2)		2.0		
15	Endosulfan (sum of isomers and endosulfan sulphate)		3.0		
16.	Endrin		0.05		
17	Ethion		2.0		
18.	Fenitrothion		0.5		
19	Fenvalerate		1.5		
20.	Fonofos		0.05		
21.	Heptachlor (sum of heptachlor and heptachlor epoxide)		0.05		
22.	Hexachlorobenzene		0.1		
23	Hexachlorocyclohexane isomers (other than y)		0.3		
24.	Lindane (y-hexachlorocyclohexane)		0.6		
25	Malathion		1.0		
26.	Methidathion		0.2		
27.	Parathion		0.5		
28.	Parathion-methyl		0.2		
29	Permethrin		1.0		
30	Phosalone		0.1		
31.	Piperonyl butoxide		3.0		
32.	Pirimiphos-methyl		4.0		
33.	Pyrethrins (sum of)		3.0		
34.	Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)		1.0		

Table 6: Aflatoxin's Analysis

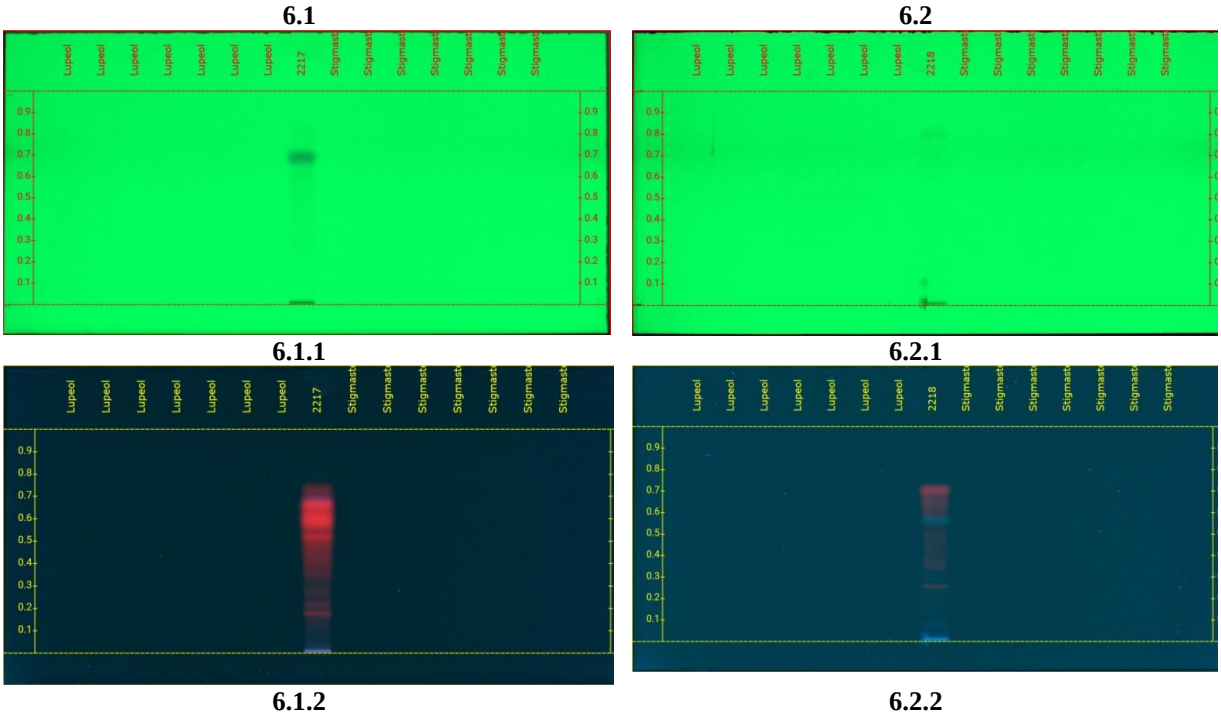
S. No.	Aflatoxin Type	Result (In seed and leaf sample)	Permissible Limit (µg/kg)	Method Used	Reference
1	Aflatoxin B1	Below the Detection limit	NMT 2 ppb	AOAC 991.31	API Part-I, Vol-II
2	Total Aflatoxins (B1 + B2 + G1 + G2)		NMT 5 ppb		

HPTLC profiling

CAMAG HPTLC instrument, syringe (100 µL), and glass twin trough developing chamber were used for the HPTLC profiling of leaves and seeds of *C. sativa* with marker compounds Lupeol and Stigmasterol. Methanolic extracts (10 µL) of leaves and seeds of *C. sativa* and methanolic standard solutions (5 µL) of lupeol and stigmasterol were applied on an aluminum plate pre-coated with silica gel 60 F254 of 0.2mm thickness (Merck, India) using an applicator CAMAG Linomat-5 and software Vision CATS 2.5. The plate was developed in a glass twin through chamber pre-saturated (for 15min) with the mobile phase. The developed plate was visualized at 254 and 366nm by using a CAMAG visualizer. The plate was derivatized with anisaldehyde-sulphuric acid reagent and heated at 105°C till the development of visible spots. The visible spots were captured by using CAMAG TLC Visualizer 2. The Rf values and densitograms were recorded by using the CAMAG Vision CATS 2.5 software, and the final report was generated. Images were captured by placing the plates in a photo-documentation chamber, and Rf values were recorded using Vision CATS 2.5 software.

Silica gel 60 F254 (Merck) was used as the stationary phase for TLC, and Ethyl acetate: Formic acid (7.0: 3.0: 0.2 v/v/v) was used as the developing solvent. The chamber was saturated for 15 min. at 25±2°C. A 100mg/mL methanolic solutions of leaves and seeds of *C. sativa* were prepared. To prepare the methanolic solutions, 100 mg of the sample was dissolved in 100 mL of methanol, sonicated for 10min at 25°C, and then centrifuged at 10,000 rpm for 5min. The supernatant was then kept in glass vials for HPTLC analysis.[12,13] The standard solution of lupeol and stigmasterol, at a concentration of 1mg/mL, was prepared in methanol. The HPTLC findings and spots of leaf and seed extracts of C. *sativa* are shown in Figure 6 and Table 7.

Figure 6: HPTLC findings of leaf and seed extract



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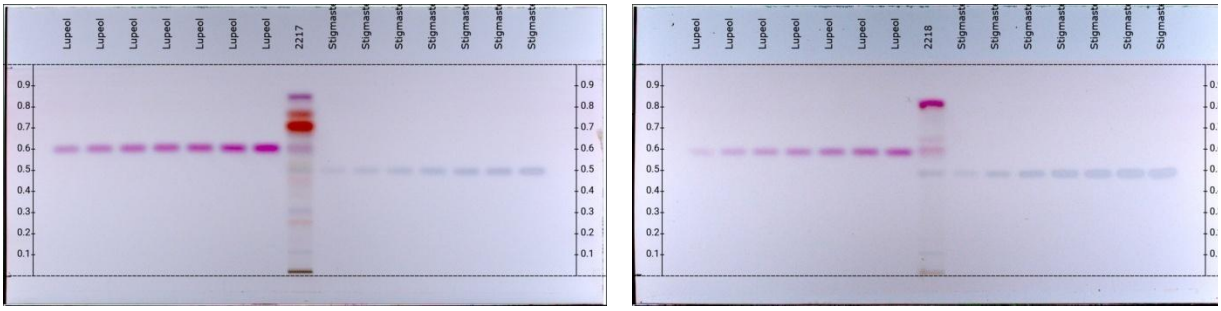

6.1.3 **6.2.3**

Figure 6: 6.1: Leaf extract; 6.1.1: Developed (At 254nm), **6.1.2:** Developed (At 366nm), and 6.1.3: Daylight. **6.2: Seed extract; 6.2.1:** Developed (At 254nm), **6.2.2:** Developed (At 366nm) and **6.2.3:** Daylight

Table 7: Rf values of seed and leaf samples at various wavelengths;

Sr. no.	Wavelength	Leaf extract (Rf)	Standards		Seed extract (Rf)	Standards	
			Lupeol	Stigmasterol		Lupeol	Stigmasterol
1	At 254nm	0.010	---	---	0.010	---	---
		0.691	---	---	0.081	---	---
		---	---	---	0.797	---	---
2	366nm	0.007	---	---	0.008	---	---
		0.177	---	---	0.089	---	---
		0.211	---	---	0.253	---	---
		0.520	---	---	0.537	---	---
		0.595	---	---	0.621	---	---
		0.660	---	---	0.687	---	---
3	White light	0.018	---	---	0.010	---	---
		0.115	---	---	0.103	---	---
		0.254	---	---	0.482	---	0.480
		0.307	---	---	0.597	0.584	---
		0.501	---	0.501	0.814	---	---
		0.605	0.606	---	---	---	---
		0.710	---	---	---	---	---
		0.764	---	---	---	---	---
		0.847	---	---	---	---	---

Microbial overload: The assessment of total microbial overload is observed to be within the standard permissible limit. (Table 8)

Table 8: Total microbial overload:

Sr. N	Test	Observation		Standard value	Standard reference
		Leaf	Seeds		
1.	Total Bacterial Count	$1x10^2$ CFU/gram*	$2x10^3$ CFU/gram*	$1x10^5$ CFU/gram*	API Part-1, Vol-I
2.	Total Fungi/Yeast Count	$1x10^2$ CFU/gram*	$1x10^2$ CFU/gram*	$1x10^3$ CFU/gram*	

Discussion:

The present study provides the physicochemical and phytopharmacognostical evaluation of the leaves and seeds of *C. sativa*, which is essential for the accurate authentication, identification, and quality assurance of this therapeutically significant plant material. Such detailed investigations play a vital role in developing

standardized profiles of the plant that support not only regulatory compliance but also ensure the safety, efficacy, and consistency of herbal preparations.

Microscopic analysis of the leaf samples revealed numerous distinct diagnostic features, including simple and glandular trichomes, parenchymatous cells containing cluster crystals. Notably, glandular trichomes are recognized for their active role in the synthesis and secretion of cannabinoid and terpenoid compounds, which are widely known for their therapeutic potential in alleviating inflammation, managing pain, and treating neurological disorders.[14] These trichomes play a key role in the plant's pharmacological activity and are frequently used as taxonomic and quality control markers for many drug species.[4]

In contrast, the seed samples demonstrated the presence of aleurone grains, spool cells, oil globules, fibers, distinct parenchyma, and epidermal cells. The occurrence of oil globules and aleurone grains is particularly significant, as it indicates that the seed is a rich source of proteins and essential fatty acids, thereby highlighting the nutritional and therapeutic relevance of *C. sativa* seeds[5]. Due to its balanced omega-6 to omega-3 fatty acid ratio in the seed oil composition, it is also valued as a functional food in many parts of the country.[15]

Preliminary phytochemical screening further highlighted the medicinal value of *C. sativa*. Both leaves and seeds were found to contain alkaloids, flavonoids, tannins, phenols, and saponins as bioactive compounds known for their robust antimicrobial, antioxidant, and anti-inflammatory etc. properties.[16,6] Interestingly, glycosides were identified exclusively in the leaf extracts, suggesting that the aerial parts of the plant possess a richer phytochemical profile with enhanced antioxidant capabilities.[4,7] Meanwhile, proteins were identified in the seeds, strengthening their potential role in dietary supplementation and therapeutic nutrition.[5] The physicochemical parameters of both the plant parts, including moisture content, total ash value, and extractive values, were all within the acceptable pharmacopoeial limits, accentuating the high quality and stability of the raw materials.[11] Physicochemical evaluation of *C. sativa* leaves is within the standard permissible limit. However, such parameters for evaluating *C. sativa* seeds are not available in the Ayurvedic Pharmacopoeia of India. Thus, the analytical findings of *C. sativa* seed can be considered as a standard for further research.

Both the leaf and seed samples of *C. sativa* were also analyzed for safety and quality parameters in accordance with the standards prescribed in the Ayurvedic Pharmacopoeia of India (API). The assessment included the determination of heavy metals, aflatoxins, pesticide residues, and microbial contamination. Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were analyzed using standard pharmacopoeial methods (USP<233>). All the heavy metals were found to be within the permissible limits set by the USP, viz.: not more than 10 ppm for lead, 0.3 ppm for cadmium, 1.0 ppm for arsenic, and 1.0 ppm for mercury. [17,18] This confirms that the plant sample is safe with respect to toxic metal contamination.

Additionally, aflatoxins, including aflatoxin B1, were analyzed in accordance with the guidelines of modified AOAC 991.31, which stipulate that total aflatoxins should not exceed 20 µg/kg, and aflatoxin B1 should not exceed 10 µg/kg. [17] The results showed that the aflatoxins were below the detection limit in both leaf and seed samples of *C. sativa,* which indicates full compliance with established safety limits. Pesticide residue analysis, conducted using AOAC official methods 2007.01, revealed that all residues were below the maximum residue limits (MRLs). These findings confirm that the both the samples are free from hazardous levels of agricultural chemicals.

HPTLC profiling is a reliable, flexible, and cost-efficient technique for herbal drug analysis, used for the identification, determination of impurities, and quantitative assessment of active substances.[19] The leaf extract of the *C. sativa* shows the two presence of spots at 254nm 0.010,0.691 total six spots at 366nm as 0.007, 0.177, 0.211, 0.520, 0.595, 0.660 and in day light total nine spots are as 0.018, 0.115,0.254, 0.307, 0.501, 0.605, 0.710, 0.764, 0.847 in which 0.606 and 0.501 matches with the lupeol and stigmasterol respectively where as in seed sample at 254 nm three spots are there as 0.010, 0.081, 0.797 at 366nm total six spots as 0.008, 0.089, 0.253, 0.537, 0.621, 0.687 and in day light total five spots are as 0.010, 0.103, 0.482, 0.597, 0.814 are present in which 0.584 and 0.480 matches with the lupeol and stigmasterol respectively.

Microbial load was assessed to ensure the hygienic quality of the plant materials. According to the Ayurvedic Pharmacopoeia of India (API), the microbial limits for raw herbal drugs specify that the total aerobic microbial count should not exceed 1×10^5 CFU/gram, and the total yeast and mold count should not exceed 1×10^3

CFU/gram. In the present study, the total aerobic bacterial count was found to be 1×10^2 CFU/gram in leaf samples and 2×10^3 CFU/gram in seed samples. The total yeast and mold count was 1×10^2 CFU/gram in both leaf and seed samples. These values come within the permissible limits, indicating good microbiological quality and appropriate post-harvest handling of the raw plant material.

Conclusion:

The findings of this study affirm the pharmacognostic distinctiveness, chemical richness, and safety of *C. sativa* leaves and seeds. The samples meet the prescribed standards of the Ayurvedic Pharmacopoeia of India in terms of identity, purity, and quality. These results provide strong foundational evidence for the safe use of *C. sativa* in all herbal and nutraceutical formulations, supporting its continued development as a regulated therapeutic plant with both medicinal and nutritional relevance.

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