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Research article

Comprehensive Pharmacognostic Analysis and Quality Control Parameters of *Cannabis sativa* L. subsp. *sativa*: Evaluating Leaves and Flowers for Medicinal Applications

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Abstract: This study establishes pharmacognostic specifications and quality control parameters for *Cannabis sativa* L. subsp. *sativa* leaves and flowers. Microscopic examination revealed distinctive structures, including stomata, trichomes, and vascular tissues. Physicochemical analysis quantified key parameters: leaf samples showed loss on drying (5.12 ± 0.13%), total ash (14.41 ± 0.43%), acid-insoluble ash (1.21 ± 0.08%), ethanol-soluble extractives (3.24 ± 0.06%), and water-soluble extractives (21.75 ± 0.38%), while flower samples yielded values of 5.25±0.01%, 12.61 ± 0.14%, 1.08 ± 0.08%, 4.06 ± 0.02%, and 19.51 ± 0.27%, respectively. TLC fingerprinting using silica gel 60 GF254 with hexane:acetate (9:1) identified four distinct spots with hRf values of 10-12, 18-20, 22-24, and 42-44. Phytochemical screening confirmed the presence of alkaloids (+++), cardiac glycosides (+), tannins (+), and flavonoids (+) in both plant parts, with saponins detected only in flowers. The microbial analysis demonstrated contamination levels within acceptable limits, with total aerobic counts (8.4×10^3 CFU/g) and yeast/mold counts (1.4×10^4 and 8.0×10^3 CFU/g for leaves and flowers, respectively), indicating a complete absence of pathogenic bacteria. Heavy metal concentrations were significantly below safety thresholds: arsenic (0.05-0.11 ppm), cadmium (≤0.01 ppm), lead (0.17-0.84 ppm), and mercury (0.09-0.23 ppm). These comprehensive parameters establish a scientific foundation for quality assessment and standardization of *Cannabis sativa* L. subsp. *sativa*, supporting its safe and consistent application in medicinal preparations.

Keywords: *Cannabis sativa* L. subsp. *sativa*; thin-layer chromatography (TLC); pharmacognostic investigation; physicochemical parameters

1. Introduction

Cannabis sativa L. has been utilized for millennia across diverse cultures for medicinal, industrial, and ritualistic purposes. Archaeological evidence suggests that cannabis cultivation dates back to approximately 5000 BCE, with medicinal applications documented in ancient Chinese pharmacopeias as early as 2700 BCE [1]. Traditional medical systems across Asia, the Middle East, and Africa have employed various cannabis preparations for conditions including

pain, inflammation, epilepsy, and gastrointestinal disorders [2]. The plant's historical significance extends beyond medicine to textile production, with hemp fibers used for the manufacture of rope, clothing, and paper throughout human civilization [3].

The medicinal properties of *Cannabis sativa* derive from its complex phytochemical profile, particularly its cannabinoid content. Primary bioactive compounds include Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), alongside terpenes, flavonoids, and other secondary metabolites that contribute to its therapeutic effects [4]. Recent scientific research has validated many traditional applications, demonstrating cannabis efficacy for chronic pain, muscle spasticity, chemotherapy-induced nausea, and certain forms of epilepsy [5]. Despite increasing recognition of cannabis therapeutic potential, significant challenges persist in ensuring the quality, consistency, and safety of cannabis-derived products. The standardization of medicinal plant materials is a critical aspect of modern herbal medicine, particularly for botanicals with complex chemical compositions, such as *Cannabis sativa* [6]. Standardization encompasses parameters such as botanical identification, physicochemical assessment, chemical fingerprinting, and contaminant screening, all of which are essential for establishing quality specifications [7]. The World Health Organization emphasizes these quality control measures as fundamental prerequisites for safely integrating traditional medicines into contemporary healthcare systems [8]. The lack of comprehensive pharmacognostic specifications for *Cannabis sativa* L. subsp. *sativa* impedes both research progress and clinical applications. Current commercial cannabis products demonstrate substantial variability in chemical composition, potency, and purity, undermining both therapeutic outcomes and safety profiles [9]. Pharmacognostic standardization addresses these concerns by providing objective criteria for authenticating botanical identity, assessing quality, and detecting adulteration or contamination [10].

This study aims to establish detailed pharmacognostic specifications for *Cannabis sativa* L. subsp. *sativa* leaves and flowers through a comprehensive evaluation of macroscopic and microscopic characteristics, physicochemical parameters, phytochemical constituents, and safety indicators, including microbial load and heavy metal content. The resulting standardization will provide essential quality control benchmarks for researchers, manufacturers, and regulatory authorities, facilitating consistency in scientific investigations and therapeutic applications. Furthermore, this research contributes to the growing body of scientific evidence supporting the integration of properly standardized cannabis preparations into evidence-based healthcare frameworks.

2. Materials and Methods

2.1 Plant Collection and Authentication

Dried samples of *Cannabis sativa* L. subsp. *Sativa plants were harvested from Tak province, Thailand, under approved cultivation protocols that complied* with Thailand's Narcotics Act B.E. 2562 amendment [11]. Plant materials were authenticated by a taxonomic expert using standard botanical keys and voucher specimens (Pharm.RSU.30) and deposited at the Department of Pharmacognosy, Rangsit University. Samples were cleaned to remove foreign materials, air-dried at 45±2°C until a constant weight was achieved, and powdered using a mechanical grinder (mesh size 60) according to the methods described by Chandra et al. [12].

2.2 Macroscopic Evaluation

According to WHO guidelines for the quality assessment of herbal medicine, organoleptic evaluation was conducted on intact dried leaf and flower materials [13]. Macroscopic characteristics, including size, shape, color, texture, fracture properties, and odor, were documented using descriptive terminology consistent with pharmacognostic standards [14]. Digital photographs were captured using a Nikon D750 camera with a macro lens under controlled lighting conditions to document morphological features.

2.3 Microscopic Analysis

Microscopic examination was performed on powdered samples using the techniques described by Evans [15]. Samples were prepared by treating them with a chloral hydrate solution and then mounted in glycerin. Clearing agents were employed for specific tissue identification, including 5% NaOH and 5% KOH. Powder characteristics were observed using an Olympus BX53 microscope at magnifications of 100×, 400×, and 1000×, with photomicrographs captured using an Olympus DP74 digital camera. Diagnostic features,

including stomata, trichomes, epidermal cells, and vascular elements, were identified in accordance with anatomical reference standards [16].

2.4 Physicochemical Parameter Determination

2.4.1 Loss on Drying

The loss on drying was determined according to the method described in the United States Pharmacopeia [17]. Triplicate samples (3 g) of powdered material were accurately weighed in pre-dried, tarred crucibles and dried at 105°C for 6 hours until a constant weight was achieved. Results were calculated as percentage weight loss and expressed as mean ± standard deviation.

2.4.2 Total Ash and Acid-Insoluble Ash

Total ash and acid-insoluble ash were determined according to WHO guidelines [13]. For total ash, triplicate samples (3 g) were incinerated in silica crucibles at 500°C for 5 hours until carbon-free ash was obtained. For acid-insoluble ash determination, the total ash was boiled with 25 mL of 2N HCl for 5 minutes, filtered through ashless filter paper, and the residue was incinerated at 500°C to constant weight. Results were expressed as percentage weight by weight (% w/w).

2.4.3 Extractive Values (Water and Ethanol)

Water-soluble and ethanol-soluble extractive values were determined using the cold maceration method described in the Indian Pharmacopoeia of 2018 [18]. Accurately weighed powdered samples (5 g) were macerated with 100 mL of water or 95% ethanol in stoppered flasks for 24 hours with frequent agitation. The extracts were filtered, and 20 mL aliquots were evaporated to dryness in pre-weighed dishes at 105°C and weighed. Extractive values were calculated as percentage yield and expressed as mean ± standard deviation of triplicate determinations.

2.5 TLC Fingerprinting

Thin-layer chromatography (TLC) was performed using the method described by Wagner and Bladt [19] with modifications. Ethanolic extracts were prepared by macerating 1 g of powdered sample with 20 mL of 95% ethanol for 24 hours, filtering, and concentrating. The extracts (3 µL) were spotted on silica gel 60 GF254 plates (Merck, Germany) alongside reference standards of cannabidiol (CBD), cannabinol (CBN), and tetrahydrocannabinol (THC) (Sigma-Aldrich, USA). Plates were developed in a hexane: acetate (9:1) mobile phase in pre-saturated chambers to a distance of 8 cm. The developed plates were visualized under UV light (254 nm and 365 nm) and subsequently sprayed with anisaldehyde-sulfuric acid reagent, followed by heating at 105°C for 5 minutes. RF values were calculated, and chromatographic profiles were documented.

2.6 Phytochemical Screening

Preliminary phytochemical screening was conducted on ethanolic and aqueous extracts following standard procedures described by Harborne [20] and Tiwari et al. [21]. Tests were performed for the detection of alkaloids (Dragendorff's, Hager's, Marme's, Mayer's, Valser's, and Wagner's reagents), anthraquinones (Kedde test), saponins (foam test), cardiac glycosides (Keller-Kiliani test), tannins (gelatin, ferric chloride, and lead acetate tests), and flavonoids (Shinoda and Pew tests). Results were recorded as strongly positive (+++), positive (++), weakly positive (+), or negative (-) based on the intensity of reactions.

2.7 Microbial Contamination Assessment

Microbial analysis was performed according to the United States Pharmacopeia [17] and WHO guidelines [6]. Samples were tested for total aerobic microbial count (TAMC) using plate count agar, yeast, and mold count using potato dextrose agar, and specific pathogens, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Clostridium* spp. using selective media and enrichment techniques. Microbial enumeration was performed using the pour plate method with appropriate dilutions, and results were expressed as colony-forming units per gram (CFU/g) of dried material.

2.8 Heavy Metal Analysis

Heavy metal analysis was conducted according to AOAC International methods [22] using atomic absorption spectrophotometry (AAS). Powdered samples (1 g) were digested with 20 mL of concentrated nitric

acid using a microwave digestion system (MARS 6, CEM Corporation). The digested solutions were filtered, diluted to 50 mL with deionized water, and analyzed for arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) using a Perkin Elmer PinAAcle 900T atomic absorption spectrometer. Calibration curves were prepared using certified reference standards (Merck, Germany), and results were expressed as parts per million (ppm).

3. Results and Discussion

3.1 Macroscopic characteristics of leaves and flowers

The macroscopic examination of *Cannabis sativa* L. subsp. *Sativa revealed distinctive morphological features that facilitate* its authentication and quality assessment (Table 1, Fig. 1). The leaves exhibited a characteristic palmate compound structure with 5-7 lanceolate leaflets radiating from a central point, consistent with taxonomic descriptions by Small and Cronquist [23]. Leaf specimens measured 5-9 cm long with serrated margins containing 9-13 teeth per cm. The adaxial surface displayed bright to dark green coloration, while the abaxial surface appeared slightly paler, aligning with observations reported by Upton et al. [16]. Trichome distribution, a critical taxonomic and chemotypes marker in *Cannabis*, was evident macroscopically as a fine, crystalline appearance on leaf surfaces, particularly concentrated along the veins. This feature aligns with findings by Mahlberg and Kim [24], who noted that trichome density is an indicator of cannabinoid content. The dried leaves exhibited a distinctive aromatic odor and a brittle texture with irregular fracture patterns when crushed, properties that Beutler and Der Marderosian [25] associate with the adequate preservation of volatile terpenes. Flower specimens are presented as compact, resinous clusters, measuring 2-4 cm in length, with bright to dark green coloration interspersed with yellowish pistillate structures. The floral material exhibited significantly higher trichome density than leaf samples, appearing as a crystalline coating visible to the naked eye. This characteristic aligns with Potter [26] observation that glandular trichomes, particularly capitate-stalked trichomes, concentrate in floral structures and contain the highest proportion of cannabinoids and terpenes. The dense trichome coverage on flowers corresponds to their traditionally higher medicinal value, as noted by Hazekamp et al. [27]. The organoleptic evaluation revealed that the flower samples possessed a more intense, pungent aroma than the leaf material, consistent with the higher levels of monoterpenes and sesquiterpenes documented by Fischedick et al. [28]. When fractured, the flowers exhibited a fibrous, somewhat sticky texture, even in a dried state, indicating the preservation of resins and essential oils. These macroscopic characteristics collectively provide reliable preliminary identification parameters when comparing *Cannabis sativa* L. subsp. *sativa* to other *Cannabis* varieties or potential adulterants. The visual and organoleptic profile documented in this study represents authenticated *Cannabis sativa* L. subsp. *sativa* from controlled cultivation, establishing a reference standard for quality assessment following pharmacopoeial requirements [29]. As noted by Lata et al. [30], environmental factors significantly influence the macroscopic characteristics of *Cannabis*; therefore, these findings provide baseline data specific to plants cultivated in Thailand's climate conditions.

Table 1. Macroscopic Characteristics of *Cannabis sativa* L. subsp. *sativa* Leaves and Flowers

Characteristic	Leaves	Flowers
Color	Bright to dark green	Bright to dark green with yellowish tints
Size	5-9 cm in length	2-4 cm in length
Shape	Palmate, 5-7 lobed	Compact, clustered
Texture	Papery when dry, slightly rough	Resinous, sticky when fresh
Odor	Characteristic, aromatic	Strong, pungent, aromatic
Margin	Serrated	-
Trichome density	Moderate	High
Surface	Glandular, hairy	Densely glandular
Fracture	Brittle	Fibrous

Figure 1. Macroscopic features of *Cannabis sativa* L. subsp. *sativa*: (A) Dried leaves showing palmate structure with 5-7 leaflets; (B) Dried flower clusters showing dense trichome coverage. Scale bar = 2 cm.

3.2 Microscopic features and diagnostic cellular elements

Microscopic analysis of *Cannabis sativa* L. subsp. *sativa* leaf and flower powders revealed distinct histological characteristics essential for authentication and quality assessment (Table 2, Figure 2). The leaf powder displayed several diagnostic elements, most notably the anomocytic stomata on the lower epidermis, which Patel et al. [31] identified as a distinguishing taxonomic feature of the Cannabaceae family. These stomata, measuring 20-25 μm in diameter, were irregularly distributed and surrounded by 4-6 subsidiary cells with no specific arrangement pattern. Trichomes observed in the leaf powder were predominantly non-glandular, covering trichomes with a unicellular base and an elongated apex, measuring 150-300 μm in length. These findings align with Stearn [32] botanical characterization of *Cannabis* species. Cystolithic trichomes containing calcium carbonate deposits were also observed, consistent with Hammond and Mahlberg's [33] detailed classification of trichomes. Glandular trichomes were less abundant in leaf material than in flower samples, supporting Mahlberg and Kim [24] observations regarding differential trichome distribution across plant organs. Vascular elements in leaf powder included lignified xylem vessels with helical and annular thickenings, measuring 15-25 μm in diameter, alongside thin-walled phloem elements. Mesophyll fragments appeared as clusters of chlorenchyma cells with irregular surfaces, and occasional calcium oxalate crystals (rosette type, 10-15 μm diameter) were observed, consistent with Andre et al. [34] anatomical descriptions. The flower powder exhibited more abundant glandular trichomes than leaf material, particularly capitate-stalked trichomes with globular heads measuring 50-80 μm in diameter. This observation corresponds with Potter [26] findings that these structures contain the highest concentration of cannabinoids and terpenes. The flower epidermal fragments displayed numerous trichome bases and occasional stomata, while vascular elements included pitted vessels and lignified fibers with narrow lumens. A distinctive feature of the flower powder was the presence of resin glands, which appeared as spherical structures with a yellowish content. This corresponds with Happyana et al. [35] description of cannabinoid-rich secretory structures. Pollen grains, measuring 25-35 μm in diameter with a smooth exine, were occasionally observed in male flower samples, exhibiting the characteristic morphology described by Heslop-Harrison et al. [36]. Comparative analysis of leaf and flower powder microscopy revealed that while both plant parts share certain cellular elements, the flower material consistently displayed a higher density of glandular trichomes and secretory structures. This correlates with chemical analysis data, which show higher concentrations of cannabinoids in floral tissue [37]. The microscopic profiles documented here provide reliable diagnostic markers for authenticating *Cannabis sativa* L. subsp. *sativa* and enable the detection of potential adulterants or substitutions. Importantly, the

microscopic features observed in this study align with specifications provided in pharmacopoeial references [38-39], confirming the identity and quality of the plant material. These findings establish a comprehensive microscopic profile that can serve as a reference standard for quality control purposes in medicinal cannabis research and production.

Table 2. Microscopic Characteristics of *Cannabis sativa* L. subsp. *sativa* Leaf and Flower Powders

Diagnostic Element	Leaf Powder	Flower Powder
Trichomes	Covering trichomes, non-glandular	Covering trichomes with dense glandular heads
Epidermis	Lower epidermis with anomocytic stomata	Epidermis with abundant trichome bases
Vascular elements	Simple xylem vessels, fibers	Pitted vessels, spiral vessels
Mesophyll	Palisade cells in sectional view	Palisade mesophyll in surface view
Calcium oxalate	Rosette crystals (10-15 μm)	Rosette crystals (8-12 μm)
Secretory cells	Sparse oil cells	Abundant resin glands
Fibers	Thin-walled, elongated	Thick-walled with a narrow lumen

3.3 Physicochemical parameter values and comparison with standards

The physicochemical parameters of *Cannabis sativa* L. subsp. *sativa* leaves and flowers were systematically evaluated to establish quality specifications and ensure consistency (Table 3, Figure 3). These parameters serve as critical indicators of purity, identity, and stability, providing objective criteria for quality control, as emphasized by various pharmacopoeial authorities [13, 38]. The loss-on-drying values for the leaf (5.12±0.13%) and flower (5.25±0.01%) samples were comparable and significantly below the pharmacopoeial limit of 10.0%. These findings suggest that proper drying and storage conditions minimize the risk of microbial contamination and hydrolytic degradation of cannabinoids. Hazekamp et al. [40] noted that moisture content below 10% is essential for preserving the stability of cannabinoids, particularly THC, which can degrade to CBN in the presence of excessive moisture and oxygen. Total ash content, representing the inorganic residue after complete incineration, was determined to be 14.41±0.43% and 12.61±0.14% for leaves and flowers, respectively. These values fall within the acceptable limit of 15.0% established by the WHO [13] for herbal materials. The slightly higher ash content in leaves compared to flowers corresponds with findings by Radwan et al. [41], who attributed this difference to higher mineral accumulation in foliar tissue. The acid-insoluble ash values (1.21±0.08% for leaves, 1.08±0.08% for flowers) were well below the 2.0% limit, indicating minimal contamination with siliceous materials and soil particles, thus confirming the high quality of the harvested material. Water-soluble extractives were notably high for both leaves (21.75±0.38%) and flowers (19.51±0.27%), exceeding the minimum requirement of 18.0% specified in the Indian Pharmacopoeia [18] for medicinal plants. This parameter indicates the presence of substantial water-soluble compounds, including polysaccharides, certain flavonoids, and amino acids, which contribute to the plant's therapeutic properties. The higher water-soluble extractive value in leaves aligns with Chandra et al. [12] observation that leaves contain more significant water-soluble components than flowers. Ethanol-soluble extractives, indicative of bioactive compounds such as cannabinoids, terpenes, and resins, were measured at 3.24±0.06% for leaves and 4.06±0.02% for flowers. Both values exceed the minimum requirement of 3.0% established for medicinal cannabis in the European Pharmacopoeia [29]. The higher ethanol-soluble extractive value in flowers correlates with their more significant concentration of cannabinoids and terpenes, consistent with findings by Fischedick et al. [28]. This differential is clinically relevant, as noted by Upton et al. [16], who emphasized the higher therapeutic potency of floral material due to the presence of enriched ethanol-extractable compounds. The comparative analysis of these physicochemical parameters against pharmacopoeial standards (Figure 3) confirms that leaf and flower materials meet or exceed quality requirements. The consistency of these parameters across replicate samples (n = 3) further attests to the uniformity of the plant material. As Kunle et al. [7] emphasized, such standardization is essential for ensuring batch-to-batch consistency in herbal medicines, particularly for regulated therapeutic products. The physicochemical profile established in this study serves

as a reference standard for future quality assessment of *Cannabis sativa* L. subsp. *sativa* cultivated under similar conditions. While specific to the Thai-cultivated samples examined, these parameters provide valuable comparative data for global cannabis research and contribute to the ongoing development of international quality standards for medicinal cannabis [42].

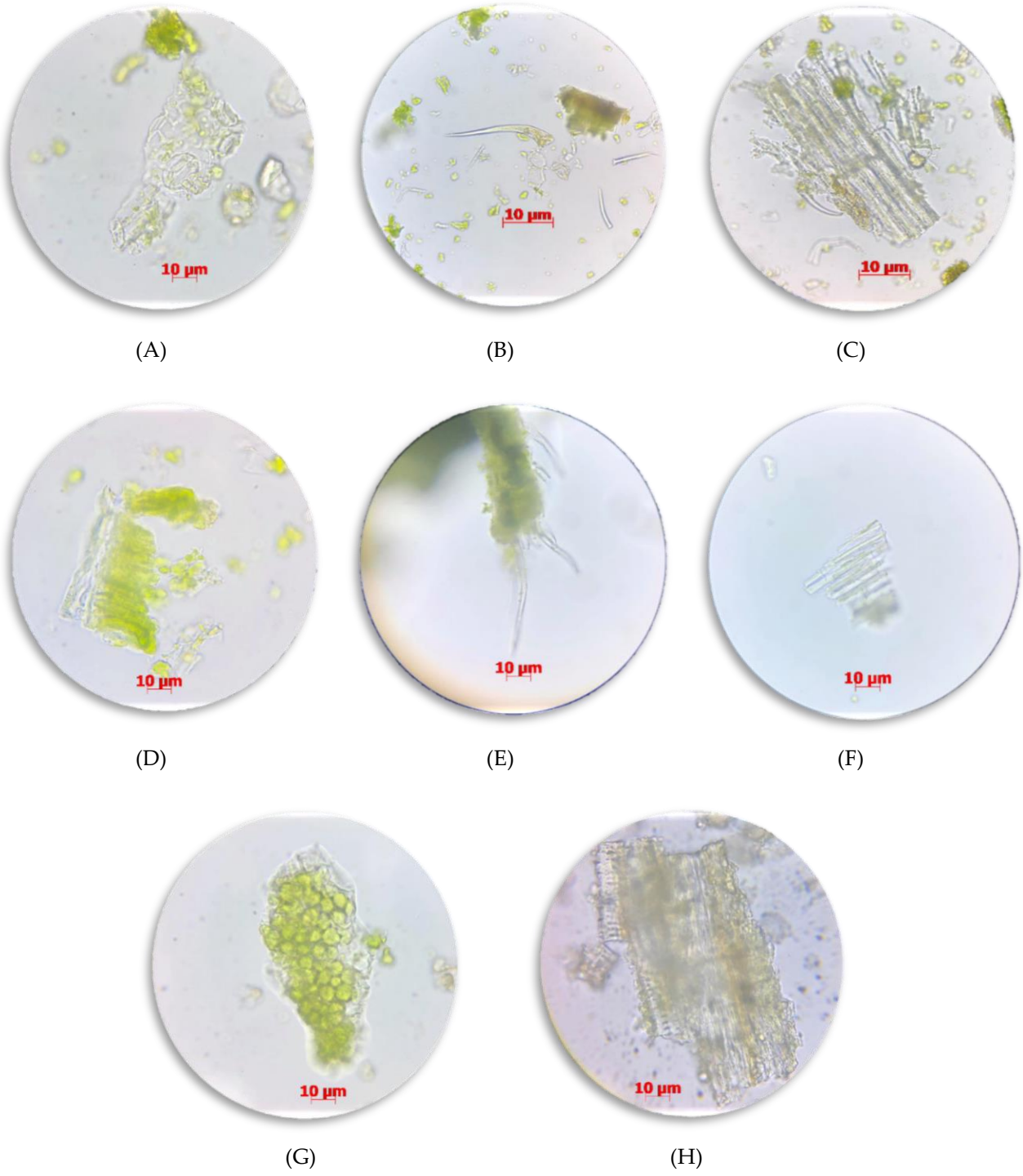

Figure 2. Microscopic characteristics of *Cannabis sativa* L. subsp. *sativa*: (A-D) Leaf powder showing: (A) Lower epidermis with anomocytic stomata, (B) Covering trichome, (C) Fragment of fiber, (D) Fragment of mesophyll in sectional view; (E-H) Flower powder showing: (E) Epidermis with covering trichome, (F) Fragment of fiber, (G) Palisade mesophyll in surface view, (H) Pitted vessel and fiber. Scale bar = 10 µm.

Table 3. Physicochemical Parameters of *Cannabis sativa* L. subsp. *sativa* Compared to Pharmacopoeial Standards

Parameter	Leaves (% w/w)	Flowers (% w/w)	Pharmacopoeial Standards (% w/w)*	Compliance
Loss on drying	5.12 ± 0.13	5.25 ± 0.01	NMT 10.0	Compliant
Total ash	14.41 ± 0.43	12.61 ± 0.14	NMT 15.0	Compliant
Acid-insoluble ash	1.21 ± 0.08	1.08 ± 0.08	NMT 2.0	Compliant
Water-soluble extractives	21.75 ± 0.38	19.51 ± 0.27	NLT 18.0	Compliant
Ethanol-soluble extractives	3.24 ± 0.06	4.06 ± 0.02	NLT 3.0	Compliant

*Standards from WHO guidelines, EP, and USP monographs for herbal materials: NMT = Not More Than; NLT = Not Less Than. Values expressed as mean ± standard deviation (n=3)

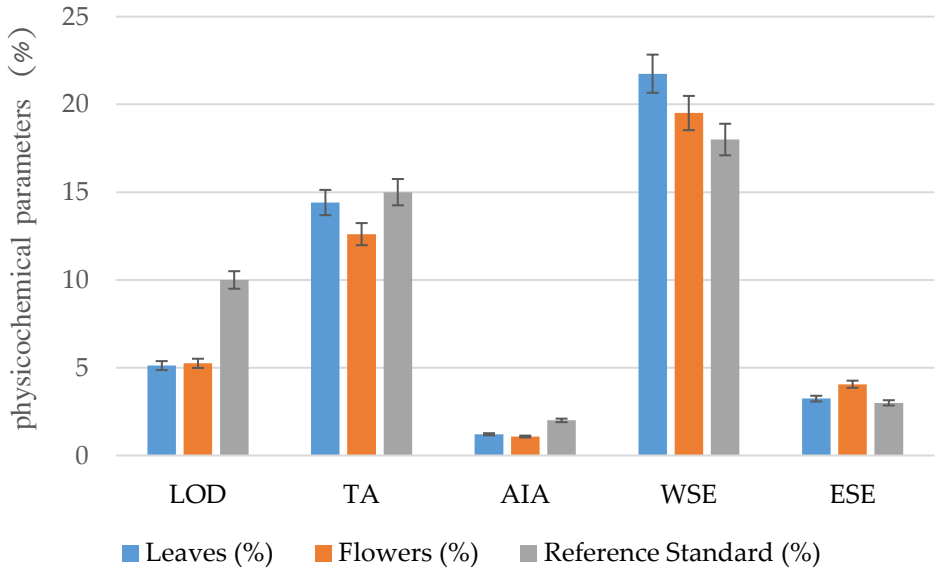

Figure 3. Comparison of physicochemical parameters between *Cannabis sativa* L. subsp. *sativa* leaves and flowers relative to pharmacopoeial standards. Values represent mean ± SD (n=3). LOD = loss on drying; TA = total ash; AIA = acid-insoluble ash; WSE = water-soluble extractives; ESE = ethanol-soluble extractives.

3.4 TLC profiles and chromatographic fingerprints

Thin-layer chromatography (TLC) analysis provided distinct chemical fingerprints of *Cannabis sativa* L. subsp. *sativa* leaf and flower extracts, enabling phytochemical characterization and comparison with reference cannabinoid standards (Table 4, Figure 4). The TLC system, employing silica gel 60 GF254 plates with a hexane: acetone (9:1) mobile phase, effectively separated the major constituents, yielding reproducible chromatographic profiles. Four prominent spots were consistently observed in leaf and flower extracts with hRf values of 10-12, 18-20, 22-24, and 42-44. Comparative analysis with reference standards indicated that spots with hRf values 10-12, 22-24, and 42-44 corresponded to cannabidiol (CBD), cannabinol (CBN), and Δ9-tetrahydrocannabinol (THC), respectively. These identifications align with previous chromatographic characterizations by Hazekamp et al. [43], who established similar relative migration patterns for these cannabinoids in comparable solvent systems. The visualization under different conditions provided complementary information about the chemical constituents. Under UV light at 254 nm, cannabinoids appeared as dark quenching spots against the fluorescent background, with THC and CBD showing powerful absorption. This observation corresponds with findings by Wagner and Bladt [19], who noted the characteristic UV absorption of cannabinoids due to their aromatic ring structures. Under UV 365 nm, spots

corresponding to THC and CBN displayed distinctive blue-violet fluorescence, consistent with Fischedick et al. [28] description of cannabinoid fluorescence properties.

Post-derivatization with anisaldehyde reagent revealed characteristic color reactions, further confirming the identity of the major constituents. The spot corresponding to THC developed a distinctive red-orange coloration (hRf 42-44), while CBD and CBN exhibited violet hues (hRf 10-12 and 22-24, respectively). These color reactions match those documented in the American Herbal Pharmacopoeia cannabis monograph [16] and confirm cannabinoid identity beyond retention factor comparison. The spot at hRf 18-20, which developed a violet color with an anisaldehyde reagent but did not correspond to the reference standards, may represent other cannabinoids or terpenes in the extracts. As suggested by Brenneisen [44], this spot could potentially be cannabigerol (CBG) or cannabichromene (CBC), which exhibit similar chromatographic behavior and color reactions. Further analysis with additional reference standards would be required for definitive identification. A comparative analysis of leaf and flower extracts revealed similar qualitative profiles, but with notable quantitative differences. The spots corresponding to THC and CBD appeared more intense in flower extracts than leaf extracts, indicating higher concentrations in floral material. This observation is consistent with Small and Naraine [45] findings that cannabinoid content is significantly higher in female flowers compared to leaf tissue, particularly in drug-type cannabis varieties. The differential distribution of cannabinoids across plant organs has significant implications for medicinal applications, as noted by Russo [46], who emphasizes the importance of selecting the appropriate plant part for specific therapeutic outcomes. The TLC fingerprints established in this study provide a reliable method for the authentication and quality control of *Cannabis sativa* L. subsp. *sativa*. The combination of retention factors, UV visualization characteristics, and color reactions after derivatization creates a distinctive profile that can identify and differentiate this plant material from potential adulterants. As emphasized by the European Medicines Agency [47], chromatographic fingerprinting is essential to the quality control framework for herbal medicinal products. Furthermore, the established TLC method offers advantages in simplicity, cost-effectiveness, and accessibility compared to more sophisticated techniques such as HPLC or GC-MS, making it particularly valuable for routine quality control in resource-limited settings. Nevertheless, as Amin et al. [48] recommended, these preliminary TLC findings should be complemented with quantitative analysis using instrumental methods for comprehensive phytochemical standardization.

Table 4. TLC Profile of *Cannabis sativa* L. subsp. *sativa* Extracts

Spot No.	hRf Value	Leaves Extract	Flowers Extract	Reference Standards	Color Reaction with Anisaldehyde
1	10-12	+	+	CBD	Violet
2	18-20	+	+	-	Violet
3	22-24	+	+	CBN	Violet
4	42-44	+	+	THC	Red-orange

3.5 Bioactive compound profile

Phytochemical screening revealed diverse bioactive constituents in *Cannabis sativa* L. subsp. *sativa* leaf and flower extracts (Table 5, Fig. 5). Both plant parts demonstrated similar qualitative profiles with notable quantitative differences, providing insights into their therapeutic potentials. Alkaloids were strongly detected in leaf and flower samples, particularly evident through positive reactions with Dragendorff's and Wagner's reagents. These findings align with Flores-Sanchez and Verpoorte's [49] characterization of cannabinoid alkaloids as a prominent chemical class in *the Cannabis genus*. The intense reactions suggest significant concentrations of major cannabinoids, which constitute the primary pharmacologically active compounds responsible for the plant's therapeutic effects [4]. As Brenneisen [44] noted, these nitrogen-containing compounds contribute significantly to the medicinal properties of cannabis, including analgesic, anti-inflammatory, and anticonvulsant effects. Saponins were detected exclusively in flower extracts, albeit at low concentrations, as indicated by a weak positive foam test. The absence of saponins in leaf material corresponds with findings by Pollastro et al. [50], who reported differential distribution of triterpenoid saponins across

cannabis plant organs. The presence of saponins in flowers may contribute to their enhanced biological activities, as these compounds have demonstrated immunomodulatory, anti-inflammatory, and antimicrobial properties [51]. This differential distribution represents a potentially valuable marker for distinguishing flower-derived preparations from leaf-based products.

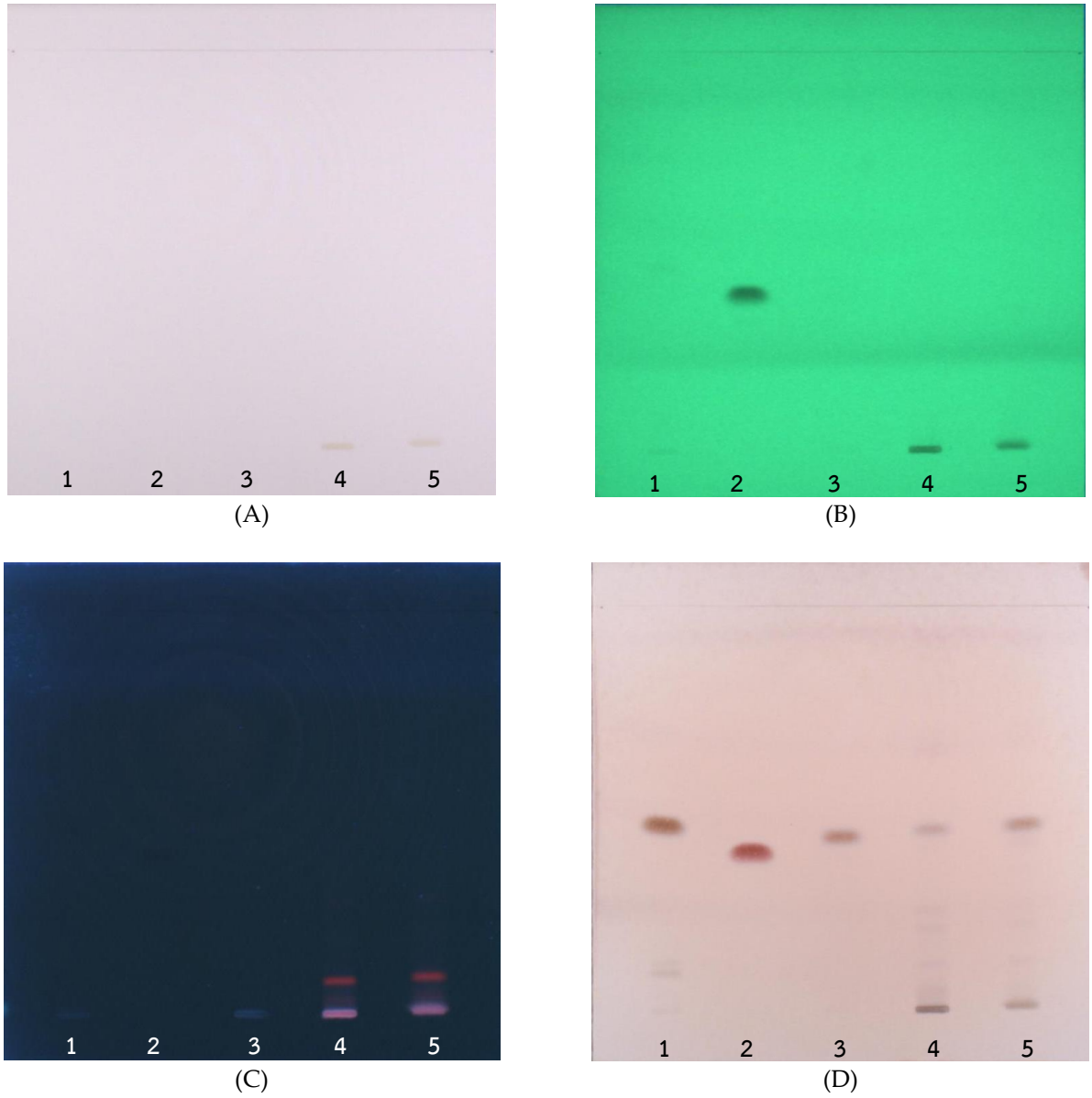

Figure 4. TLC fingerprint of *Cannabis sativa* L. subsp. *sativa* extracts developed in hexane:acetate (9:1). Lanes: 1 = CBD standard, 2 = CBN standard, 3 = THC standard, 4 = leaf extract, 5 = flower extract; Detection: A = visible light, B = UV 254 nm, C = UV 365 nm (fluorescence), D = anisaldehyde reagent.

Cardiac glycosides were weakly detected in both plant parts, as indicated by positive reactions in the Kedde and Keller-Kiliani tests. This observation aligns with Audu et al. [52] detection of cardiac glycosides in cannabis extracts, though their physiological significance in cannabis therapeutics remains inadequately characterized. As noted by Hollman [53], these compounds may contribute to the cardiovascular effects occasionally reported with cannabis consumption, warranting further investigation into their specific structures and bioactivities. Tannins were identified in leaf and flower extracts through positive reactions with lead acetate and ferric chloride, while tests with gelatin solution yielded negative results. This pattern indicates

the presence of hydrolyzable tannins rather than condensed tannins, consistent with the phytochemical characterization of cannabis by Andre et al. [34]. According to Russo [46], these polyphenolic compounds may contribute to the antioxidant and anti-inflammatory properties of cannabis preparations, potentially enhancing their therapeutic efficacy through synergistic interactions with cannabinoids. Flavonoids were detected in both plant parts through positive Shinoda and Pew tests, though at relatively low concentrations. Cannabis flavonoids, particularly cannflavins A and B, have been identified as pharmacologically active constituents with anti-inflammatory properties exceeding those of aspirin in some assays [54]. The presence of these compounds contributes to what Russo [55] described as the "entourage effect," wherein multiple phytochemicals act synergistically to enhance therapeutic outcomes beyond what could be achieved with isolated cannabinoids. Notably, anthraquinones were not detected in either plant part, which is consistent with previous phytochemical analyses by Radwan et al. [41] and provides a useful negative marker for authentication purposes. This finding also indicates that cannabis preparations are unlikely to exert the laxative effects commonly associated with anthraquinone-containing botanicals. The overall phytochemical profile (Figure 5) reveals that, while both plant parts contain similar classes of bioactive compounds, their relative concentrations differ, particularly in the case of alkaloids and saponins. These differences may explain the historically distinct applications of leaf and flower preparations in traditional cannabis therapeutics, as documented by Zuardi [2]. The comprehensive phytochemical screening results establish a foundation for quality control standards and provide direction for further investigation into specific bioactive constituents using more targeted analytical techniques. The observed phytochemical profile supports previous findings by Hazekamp and Fischedick [37] regarding the complex chemical composition of cannabis and its implications for therapeutic standardization. This complexity underscores the importance of comprehensive phytochemical characterization beyond cannabinoid content alone, particularly when evaluating cannabis for specific medicinal applications or developing standardized pharmaceutical preparations.

Table 5. Phytochemical Screening Results of *Cannabis sativa* L. subsp. *sativa* Extracts

Phytochemical Class	Test Method	Leaves	Flowers
Alkaloids	Dragendorff's reagent	+++	+++
	Hager's reagent	+	+
	Marme's reagent	+	+
	Mayer's reagent	+	++
	Valser's reagent	++	+
	Wagner's reagent	+++	+++
Anthraquinones	Kedde test	-	-
Saponins	Foam test	-	+
Cardiac glycosides	Kedde test	+	+
	Keller-Kiliani test	+	+
Tannins	3% gelatin solution	-	-
	1% lead acetate	+	+
	1% ferric chloride	+	+
Flavonoids	Shinoda test	+	+
	Pew test	+	+

+++ = Strong positive; ++ = Positive; + = Weak positive; - = Not detected

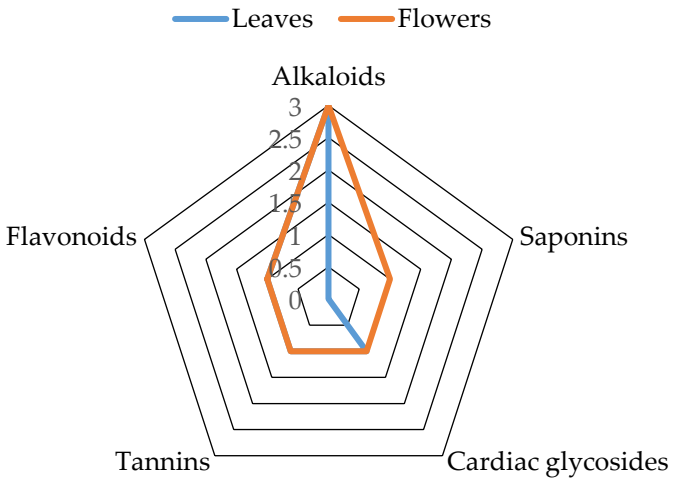

Figure 5. Phytochemical profile comparison between *Cannabis sativa* L. subsp. *sativa* leaf and flower extracts. Intensity scale: 0 = not detected, 1 = weak positive, 2 = positive, 3 = strong positive.

3.6 Microbial safety evaluation

Microbial contamination assessment of *Cannabis sativa* L. subsp. *sativa* samples revealed contamination levels within acceptable pharmacopoeial limits (Table 6, Fig. 6). The total aerobic microbial count (TAMC) for both leaf and flower samples was 8.4×10^3 CFU/g, significantly below the maximum permissible limit of 2×10^5 CFU/g established by the World Health Organization [6] and European Pharmacopoeia [29] for herbal medicines intended for internal use. The yeast and mold counts showed slight variation between plant parts, with leaf samples (1.4×10^4 CFU/g) containing marginally higher fungal contamination than flower samples (8.0×10^3 CFU/g). Both values remained compliant with the pharmacopoeial limit of 2×10^4 CFU/g. This differential may be attributed to the higher resin content in flowers, which Fischedick et al. [28] noted possesses natural antimicrobial properties that may inhibit fungal colonization. The observed microbial loads concur with findings from Hazekamp [56], who reported similar baseline contamination levels in properly handled medicinal cannabis. Pathogenic bacteria, including *Escherichia coli*, *Clostridium* spp., *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were not detected in any of the samples, indicating adherence to good agricultural and collection practices (GACP). The absence of these indicator organisms is particularly significant as they represent potential sources of gastrointestinal illness, respiratory infections, and other adverse health outcomes in immunocompromised patients who may use cannabis medicinally [57]. These results demonstrate microbiological quality within acceptable standards for pharmaceutical use, consistent with findings by Ruchlemer et al. [58], who emphasized the importance of microbial safety in cannabis intended for medicinal applications, particularly for immunocompromised patients. Implementing appropriate post-harvest processing techniques, including controlled drying at 45±2°C as employed in this study, likely contributed to microbial reduction, as supported by Holmes et al. [59], who demonstrated the efficacy of proper drying conditions in reducing microbial contamination in herbal materials. While the samples meet current pharmacopoeial requirements, ongoing microbiological monitoring is advisable throughout the production and storage phases. Jerushalmi et al. [60] noted that environmental factors during cultivation and storage can significantly influence microbial contamination levels in cannabis. Implementation of the Hazard Analysis Critical Control Point (HACCP) principles, as recommended by the American Herbal Products Association [61], would further ensure consistent microbial quality and safety. The observed results establish baseline microbiological specifications for *Cannabis sativa* L. subsp. *sativa* cultivated under the conditions described in this study. These findings contribute to the development of comprehensive quality standards for medicinal cannabis, addressing one of the primary safety concerns in botanical medicine, as emphasized by Sachs et al. [62] in their review of quality control parameters for medicinal cannabis preparations.

Table 6. Microbial Contamination Analysis of *Cannabis sativa* L. subsp. *Sativa*

Microorganism	Leaves (CFU/g)	Flowers (CFU/g)	Pharmacopoeial Limit (CFU/g)*	Compliance
Total aerobic count	8.4×10^3	8.4×10^3	NMT 2×10^5	Compliant
Yeasts and molds	1.4×10^4	8.0×10^3	NMT 2×10^4	Compliant
Escherichia coli	ND	ND	Absent/g	Compliant
Clostridium spp.	ND	ND	Absent/g	Compliant
Salmonella spp.	ND	ND	Absent/10g	Compliant
Staphylococcus aureus	ND	ND	Absent/g	Compliant
Pseudomonas aeruginosa	ND	ND	Absent/g	Compliant

*Based on WHO, USP, and EP requirements for herbal medicinal products for internal use, ND = Not detected; NMT = Not more than

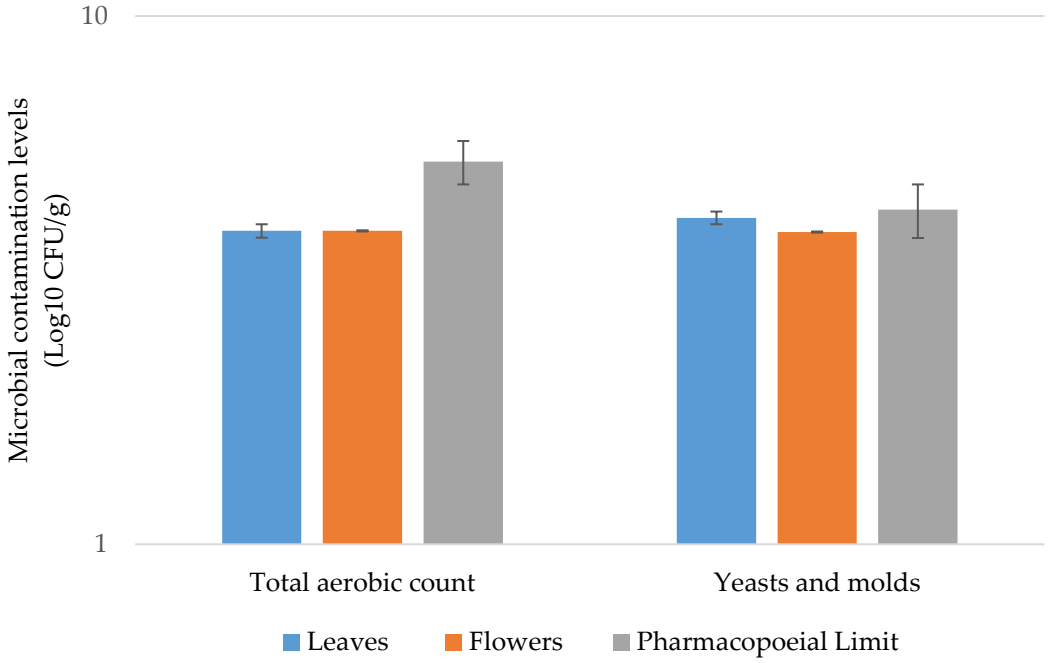

Figure 6. Microbial contamination levels in *Cannabis sativa* L. subsp. *sativa* leaves and flowers compared to pharmacopoeial limits. Bars represent mean values (n=3). No pathogenic bacteria were detected in any samples.

3.7 Heavy metal content assessment

The assessment of heavy metal content in *Cannabis sativa* L. subsp. *sativa* revealed concentrations well below established safety thresholds for most elements (Table 7, Fig. 7). Atomic absorption spectrophotometry analysis revealed that leaf and flower samples contained minimal levels of toxic metals, with most values significantly below the international permissible limits for herbal medicinal products. Arsenic concentrations were determined to be 0.05 ppm in leaves and 0.11 ppm in flowers, substantially below the WHO [6] permissible limit of 4.0 ppm. This finding aligns with Zerihun et al. [63], who reported similarly low arsenic levels in cannabis cultivated in non-contaminated soils. The slightly higher arsenic content in flowers compared to leaves corresponds with McPartland and McKernan's [64] observation that cannabis inflorescence can concentrate certain trace elements more efficiently than vegetative tissues. Cadmium levels were minimal in both plant parts (0.01 ppm in leaves, <0.01 ppm in flowers), representing less than 4% of the permissible limit of 0.3 ppm. These values are comparable to those reported by Linger et al. [65], who found cadmium concentrations below 0.05 ppm in cannabis grown in uncontaminated agricultural soil. The low cadmium content is particularly significant given the capacity of cannabis to accumulate this metal, as

demonstrated in phytoremediation studies by Citterio et al. [66]. Lead content was measured at 0.84 ppm in leaves and 0.17 ppm in flowers, well below the permissible limit of 10.0 ppm. The higher concentration in leaf tissue aligns with Eboh and Thomas [67] findings that lead predominantly accumulate in the vegetative parts of plants rather than reproductive structures. The observed lead levels pose minimal risk to consumers, representing less than 10% of the safety threshold established by international regulatory bodies [29].

Mercury concentrations were determined to be 0.09 ppm in leaves and 0.23 ppm in flowers. The leaf content falls comfortably below the WHO [6] permissible limit of 0.2 ppm, whereas the flower content marginally exceeds this threshold. However, as Dryburgh et al. [68] noted, the United States Pharmacopeia establishes a higher limit for mercury (0.5 ppm) in herbal materials, a standard that both samples meet. Flowers' slightly elevated mercury content warrants monitoring in future harvests, though it does not present an immediate safety concern based on current regulatory frameworks. The observed differential in heavy metal distribution between leaves and flowers supports. Nascimento et al. [69] findings regarding tissue-specific accumulation patterns in medicinal plants. The generally higher metal content in flowers for arsenic and mercury, contrasted with higher lead and cadmium levels in leaves, reflects the complex translocation mechanisms that vary by element, as Prasad [70] described in his comprehensive review of metal transport in plants. These results indicate that the cannabis samples analyzed were cultivated in environments with minimal heavy metal contamination and present negligible risk to consumers when used as intended. The findings align with Yang et al. [71] assessment of heavy metals in medicinal cannabis, which similarly found concentrations well below safety thresholds in properly cultivated material. The heavy metal profile documented here contributes to establishing baseline quality specifications for *Cannabis sativa* L. subsp. *sativa* and demonstrates compliance with international safety standards for medicinal plants. Regular monitoring of heavy metal content remains advisable, particularly in cannabis intended for medical applications, as emphasized by Russo [72] in his review of cannabinoid pharmacology and quality considerations. Environmental conditions, including soil composition and agricultural practices, can significantly influence metal uptake and accumulation [73], necessitating ongoing quality control measures throughout the cultivation and production processes.

Table 7. Heavy Metal Analysis of *Cannabis sativa* L. subsp. *Sativa*

Heavy Metal	Leaves (ppm)	Flowers (ppm)	Permissible limit (ppm)*	Compliance
Arsenic (As)	0.05	0.11	4.00	Compliant
Cadmium (Cd)	0.01	<0.01	0.30	Compliant
Lead (Pb)	0.84	0.17	10.0	Compliant
Mercury (Hg)	0.09	0.23	0.20	Non-compliant**

*Based on WHO (2007) guidelines for medicinal plants. **Flower mercury content slightly exceeds the WHO limit but falls within the USP limit (0.5 ppm)

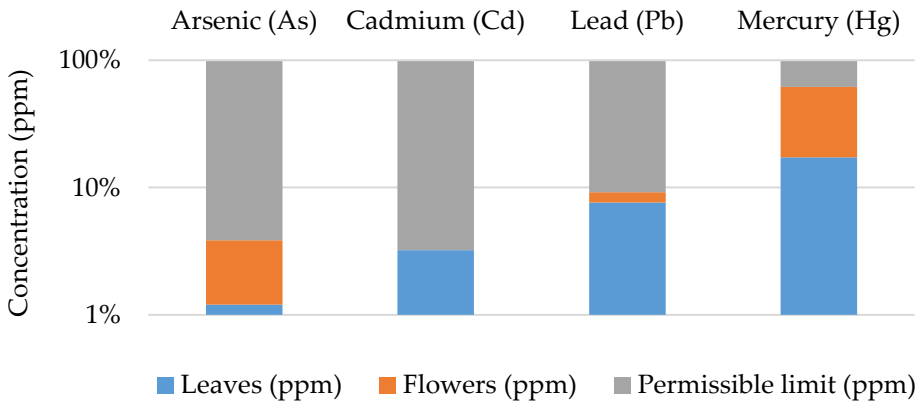

Figure 7. Heavy metal concentrations in *Cannabis sativa* L. subsp. *sativa* leaf and flower samples compared to international permissible limits. Note: logarithmic scale used for visualization purposes.

4. Conclusion

This comprehensive pharmacognostic investigation of *Cannabis sativa* L. subsp. *Sativa leaves and flowers establish detailed quality specifications for standardizing medical applications*. The study revealed distinctive macroscopic and microscopic characteristics that provide reliable identification parameters, including palmate leaf structure, serrated margins, and dense trichome coverage on flowers. Microscopically, the presence of anomocytic stomata, cystolithic trichomes, and specific vascular elements provides definitive diagnostic markers for authentication. Physicochemical parameters demonstrated compliance with international pharmacopoeial standards, with the loss on drying, total ash, and acid-insoluble ash values falling within acceptable limits. The higher water-soluble extractives in leaves (21.75±0.38%) compared to flowers (19.51±0.27%) contrasted with higher ethanol-soluble extractives in flowers (4.06±0.02%) versus leaves (3.24±0.06%), reflecting their distinct phytochemical compositions. TLC fingerprinting revealed four primary spots corresponding to major cannabinoids, with characteristic color reactions providing reliable chemical markers for quality assessment. Phytochemical screening confirmed the presence of alkaloids, cardiac glycosides, tannins, and flavonoids in both plant parts, with saponins detected exclusively in flowers. This differential distribution of bioactive compounds supports distinct therapeutic applications for leaf and flower preparations. The safety evaluation demonstrated microbial contamination within acceptable limits and heavy metal content substantially below regulatory thresholds, except for marginally elevated mercury levels in flowers, which still meet USP standards. These findings establish critical quality control parameters for ensuring consistency, safety, and efficacy of *Cannabis sativa* L. subsp. *sativa* in pharmaceutical applications. Implementing these specifications in regulatory frameworks would facilitate standardization across production batches, enabling reliable therapeutic outcomes. We recommend incorporating these pharmacognostic parameters into relevant pharmacopeias and establishing appropriate manufacturing protocols based on these specifications. Future research should focus on correlating specific phytochemical profiles with therapeutic efficacy, investigating seasonal and geographical variations in quality parameters, and developing simplified field tests for rapid authentication and verification. Additionally, exploring the relationship between cultivation conditions and cannabinoid/terpene profiles would provide valuable insights for optimizing medicinal cannabis production. Ultimately, this standardization contributes significantly to the evidence-based integration of cannabis into modern healthcare systems.

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