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Phytochemical profiles and antioxidant activities of four *Cannabis sativa* cultivars in Thailand

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Abstract. *Chokchaisiri S, Ngivprom U, Phatthanaphong P, Boon-Orn K, Wongsonthom S, Chimpalee P. 2026. Phytochemical profiles and antioxidant activities of four* Cannabis sativa *cultivars in Thailand. Biodiversitas 27 (1): d270129. https://doi.org/10.13057/biodiv/d270129.* *Cannabis sativa* produces diverse bioactive metabolites, including cannabinoids, phenolics, and flavonoids, which collectively shape its chemical and functional diversity. This study compared four cannabis cultivars cultivated under uniform conditions in Thailand (Sensi Dawg, Top Cherry Gas, Wedding Cake, and Dur Burger) to classify chemotypes and examine cultivar-dependent differences in antioxidant capacity. Ethanolic extracts were analyzed for ten cannabinoids by HPLC, together with Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activities evaluated using DPPH, ABTS, and FRAP assays. Distinct chemotypic differentiation was observed among cultivars. Dur Burger was classified as a CBD-dominant cultivar, with high CBD content, and showed the strongest activity in the ABTS assay. In contrast, Sensi Dawg, Top Cherry Gas, and Wedding Cake were THC-dominant cultivars and exhibited comparatively higher TPC and TFC levels, which were associated with stronger DPPH scavenging and ferric reducing power. Quantitative values are reported on a per-extract basis (mg per g extract). Because individual cannabinoids were not isolated for individual testing, these relationships are interpreted as associations rather than direct mechanisms; proposed links between CBD-rich profiles and ABTS activity are therefore hypotheses consistent with the present data and prior literature, not directly evaluated here. Overall, substantial phytochemical and antioxidant diversity was evident among cultivars. Variation in antioxidant performance reflected not only phenolic and flavonoid abundance but also differences in overall cannabinoid profiles, indicating contributions from multiple metabolite classes. This study addresses a gap in systematic, side-by-side profiling of Thai-grown cannabis by integrating quantitative cannabinoid analysis with multiple antioxidant assays. Key limitations include in vitro testing only, the use of ethanolic extracts from a single indoor location, and the absence of assays on isolated cannabinoids. Nevertheless, the findings provide cultivar-specific reference data to support evidence-based selection of chemotypes for pharmaceutical, nutraceutical, and functional biomaterial applications.

Keywords: Antioxidant activity, cannabinoids, *Cannabis sativa*, chemotype classification, phytochemical profiling

INTRODUCTION

Cannabis sativa L. is a plant of considerable cultural, economic, and scientific importance, with applications spanning medicinal, recreational, and industrial sectors (Bonini et al. 2018). Its biological activities are largely attributed to cannabinoids, distinctive class of terpenophenolic compounds unique to the genus. Among these, Δ^9-tetrahydrocannabinol (THC) is primarily responsible for psychoactive effects, whereas cannabidiol (CBD) has attracted increasing attention for its therapeutic potential without intoxicating properties (Iffland and Grotenhermen 2017). Genetic diversity, together with environmental influences, has led to the differentiation of cannabis into distinct chemotypes, commonly classified as THC-dominant, CBD-dominant, or intermediate types. These chemotypes differ in pharmacological properties, safety considerations, and functional applications, making chemotype classification a fundamental step for cultivar selection and product standardization (Hazekamp and Fischedick 2012). In addition to cannabinoids, *C. sativa* produces other secondary metabolites such as terpenes, phenolic acids, and flavonoids, which contribute to its chemical complexity and antioxidant properties (Russo 2011; Liguori et al. 2018). Phenolic compounds and flavonoids are well-recognized antioxidants and play important roles in radical scavenging and redox regulation. However, how these metabolite classes vary across different cannabinoid chemotypes and collectively influence antioxidant behavior remains incompletely understood, particularly when multiple chemical classes coexist within the same extract.

Phytochemical expression in cannabis is strongly influenced by environmental and agronomic factors, including light regime, temperature, humidity, nutrient availability, and cultivation system. Controlled indoor cultivation systems are frequently employed to reduce environmental variability and stabilize cannabinoid profiles (Rodriguez-Morrison et al. 2021). Evaluating multiple cultivars under standardized cultivation conditions is therefore essential to distinguish intrinsic, cultivar-specific phytochemical differences from environmentally driven variation.

In Thailand, the legalization of medical cannabis has stimulated rapid expansion of regulated cultivation and the

introduction of diverse foreign cultivars for research and commercial production (Government Gazette 2019). This has substantially increased the diversity of *C. sativa* germplasm cultivated in the country, contributing to a growing pool of genetic and biochemical resources (Jackson et al. 2007; FAO 2010). Although regulatory frameworks and cultivation practices have been documented (Zinboonyahgoon et al. 2021; Ministry of Public Health 2022), relatively few studies have systematically examined the phytochemical characteristics of cannabis grown in Thailand, particularly chemotype classification integrated with functional bioactivity. Existing work often focuses on cannabinoid content alone or evaluates antioxidant activity without linking it to cannabinoid profiles (Hazekamp and Fischedick 2012; Tipparat et al. 2012), limiting its relevance for targeted cultivar selection.

Compared with regional studies in Southeast Asia that primarily report cannabinoid distributions or basic bioactivity screening, comprehensive, side-by-side evaluations integrating chemotype classification with multiple antioxidant assays under controlled cultivation remain scarce. From a biodiversity perspective, chemotypic and phytochemical variation among cannabis cultivars represents an important dimension of agro-biodiversity and genetic resource diversity (Altieri 1999; Jackson et al. 2007). Distinct cannabinoid, phenolic, and flavonoid profiles not only determine functional properties but also reflect underlying genetic differentiation relevant to germplasm conservation, breeding, and sustainable utilization (Hillig and Mahlberg 2004; McPartland and Guy 2017). Antioxidant activity is of particular interest for pharmaceutical and nutraceutical applications, as oxidative stress is implicated in numerous pathological conditions (Liguori et al. 2018). Common in vitro assays, including DPPH, ABTS, and FRAP, provide complementary insights into radical scavenging and reducing capacities (Ilyasov et al. 2020). When combined with quantitative analysis of cannabinoids, phenolics, and flavonoids, these assays offer a more integrated view of how chemical diversity relates to functional bioactivity (Bonini et al. 2018; Judžentienė et al. 2023).

Therefore, a clear research gap remains in systematic, side-by-side evaluation of Thai-grown cannabis that integrates detailed cannabinoid profiling with antioxidant characterization under standardized cultivation conditions while explicitly considering chemotypic diversity as a component of bioresource diversity (CBD 1992; FAO 2010). Addressing this gap is essential for evidence-based utilization, standardized product development, and informed germplasm conservation in Thailand (Jackson et al. 2007; McPartland and Guy 2017). Accordingly, this study aimed to (i) classify four cultivars cultivated in Thailand based on quantitative profiling of major cannabinoids, (ii) compare their antioxidant capacities using complementary in vitro assays (DPPH, ABTS, and FRAP), and (iii) examine associations between chemotypes, phenolic and flavonoid contents, and antioxidant performance. The findings provide cultivar-specific reference data to support targeted selection, conservation, and value-added development of *C. sativa* for pharmaceutical, nutraceutical, and functional bioresource applications.

MATERIALS AND METHODS

Plant material

Four *Cannabis sativa* L. cultivars (Sensi Dawg, Top Cherry Gas, Wedding Cake, and Dur Burger) were collected in May 2024 from a licensed indoor cultivation facility located in Bang Kaeo Subdistrict, Mueang Samut Songkhram District, Samut Songkhram Province, Thailand. For each cultivar, three biological replicates were prepared, with each replicate consisting of pooled floral samples harvested from six independent plants, which were randomly selected across the cultivation area to minimize selection bias and to capture within-cultivar variability. All cultivars were grown under a controlled indoor environment with standardized cultivation conditions, including temperature 24-28°C, relative humidity 50-60%, and regulated photoperiods (18 h light/6 h dark during the vegetative stage and 12 h light/12 h dark during the flowering stage). Plants were harvested 8-9 weeks after the onset of flowering, corresponding to the mature milky-amber trichome stage, to ensure consistency in phytochemical composition. Taxonomic identification was confirmed, and voucher specimens were deposited at the Department of Cannabis Health Sciences, College of Allied Health Sciences, Suan Sunandha Rajabhat University, under voucher numbers SSRU-SC066 (Sensi Dawg), SSRU-SC067 (Top Cherry Gas), SSRU-SC068 (Wedding Cake), and SSRU-SC069 (Dur Burger).

Extraction

Fresh cannabis flowers were air-dried in the dark at room temperature 25-28°C for seven days, subsequently oven-dried at 40°C until constant weight was achieved. The dried materials were ground into a fine powder using a stainless-steel grinder. For extraction, 50 g of powdered material from each biological replicate was extracted with 95% ethanol using ultrasonic-assisted extraction (three cycles of 25 min each). The combined extracts were filtered and concentrated under reduced pressure to obtain crude ethanolic extracts. The crude extracts were stored at -20°C, protected from light, until analyses were performed. Each biological replicate extract was independently subjected to all subsequent analyses, including cannabinoid profiling, total phenolic and flavonoid determination, and antioxidant activity assays.

High-Performance Liquid Chromatography (HPLC) for cannabinoid analysis

Cannabinoid profiling was performed using an HPLC system equipped with a C18 reversed-phase column (150 × 4.6 mm, 2.7 μm). The mobile phase consisted of 0.085% phosphoric acid in water (solvent A) and acetonitrile (solvent B), operated under gradient elution conditions. The gradient program was as follows: 70% B (0-3 min), 85% B (3-7 min), 95% B (7-8 min), followed by re-equilibration to 70% B (8-12 min). The flow rate was maintained at 1.6 mL/min, the column temperature was set at 35°C, the injection volume was 5 μL, and detection was carried out at 220 nm.

Ten cannabinoids, including CBDV, CBDA, CBGA, CBG, CBD, THCV, CBN, Δ^9-THC, CBC, and THCA, were quantified using certified reference standards. Calibration curves for all analytes showed excellent linearity (R^2 > 0.999; Table 1). Limits of Detection (LOD) and Limits of Quantification (LOQ) were determined based on signal-to-noise ratios of 3 and 10, respectively. Method repeatability was assessed by triplicate injections, yielding Relative Standard Deviations (RSDs) of peak areas below 3% for major cannabinoids. Retention times were highly stable across analytical runs (RSD < 1%). For sample preparation, 50 mg of each crude ethanolic extract was dissolved in 10 mL of methanol, vortexed, and sonicated for 15 min. The solution was allowed to settle, and an aliquot of the supernatant was diluted fivefold with methanol prior to analysis. All samples were filtered through 0.22-µm PTFE syringe filters before injection. Each sample was injected in triplicate, and cannabinoid concentrations were calculated as mean±standard deviation.

Cannabinoid contents were expressed as milligrams per gram of crude ethanolic extract (mg/g dry extract). Accordingly, the high numerical values (e.g., > 500 mg/g) reflect the relative composition of cannabinoids within the concentrated extract rather than the original plant material. Although external calibration provided robust within-study comparisons, the absence of an internal standard may limit direct inter-study comparability, as matrix effects, extraction recovery, and instrumental variability could influence absolute concentrations.

Calculation of total THC and total CBD

Total THC was calculated to account for the decarboxylation of THCA according to the following equation: Total THC = Δ^9-THC + (0.877 × THCA)
Where: 0.877 represents the molecular-weight conversion factor from cannabinoid acid to the neutral form (314.47/358.48). Total CBD was calculated analogously as:

Total CBD = CBD + (0.877 × CBDA)

Extraction recovery and the use of an internal standard were not evaluated in this study. Therefore, cannabinoid quantification was based on external calibration, and the results are interpreted as comparative rather than absolute concentrations. Chromatographic separation was achieved with consistent retention times across analytical runs, with relative standard deviations of retention times below 1%. System repeatability was confirmed by triplicate injections, yielding relative standard deviations of peak areas below 3% for major cannabinoids.

Determination of total phenolic and flavonoid contents

Total Phenolic Content (TPC) was determined by the Folin-Ciocalteu colorimetric method, following established microplate-based protocols for plant extracts (Ahmed et al. 2019; Judžentienė et al. 2023). Crude cannabis extracts were initially prepared as stock solutions at 1 mg/mL in methanol. Working solutions were then diluted to final concentrations of 1-40 ppm (1, 5, 10, 15, 20, 25, 30, and 40 ppm). This concentration range was selected based on preliminary experiments to ensure linear absorbance responses within the dynamic range of the Folin-Ciocalteu assay and to avoid signal saturation commonly observed at higher extract concentrations for phenolic-rich samples. In a 96-well microplate, 20 µL of each diluted extract was mixed with diluted Folin-Ciocalteu reagent and incubated for 5 min. Subsequently, 80 µL of 7% (w/v) sodium carbonate solution was added, and the reaction mixtures were incubated at room temperature for 30 min in the dark. Absorbance was measured at 760 nm using a microplate reader. Gallic acid was used as the reference standard, and the calibration curve was constructed over the concentration range of 5-50 µg/mL, yielding the regression equation $y = ax + b$ with a coefficient of determination (R^2) > 0.999 (Figure 1.A). A reagent blank containing methanol in place of extract was prepared for background correction. All standards and samples were analyzed in triplicate. TPC was expressed as milligrams of Gallic Acid Equivalents per gram of dry extract (mg GAE/g).

Table 1. Linear regression equations, coefficients of determination (R^2), Limits of Detection (LOD), and Limits of Quantification (LOQ) for cannabinoid standards

Standard cannabinoids	Linear equation	R^2 value	LOD (mg/L)	LOQ (mg/L)
CBDV	y = 13259.6x - 480.096	0.9996	0.14	0.43
CBDA	y = 24238.6x - 1288.65	0.9995	0.18	0.55
CBGA	y = 13098.9x - 768.586	0.9995	0.18	0.53
CBG	y = 12656.6x - 588.956	0.9996	0.20	0.59
CBD	y = 12835.8x - 565.245	0.9996	0.20	0.59
THCV	y = 12942.2x - 483.047	0.9996	0.20	0.62
CBN	y = 19782.9x - 1168.59	0.9994	0.12	0.37
Δ^9-THC	y = 12708.8x - 255.379	0.9997	0.17	0.52
CBC	y = 11972.5x - 531.893	0.9995	0.20	0.60
THCA	y = 11069.7x - 532.213	0.9994	0.21	0.64

Note: The abbreviations represent the names of standard cannabinoids: CBDV: Cannabidivarin, CBDA: Cannabidiolic acid, CBGA: Cannabigerolic acid, CBG: Cannabigerol, CBD: Cannabidiol, THCV: Tetrahydrocannabivarin, CBN: Cannabinol, Δ^9-THC: Delta-9 tetrahydrocannabinol, CBC: Cannabichromene, and THCA: Tetrahydrocannabinolic acid. LOD and LOQ were determined at signal-to-noise ratios of 3 and 10, respectively

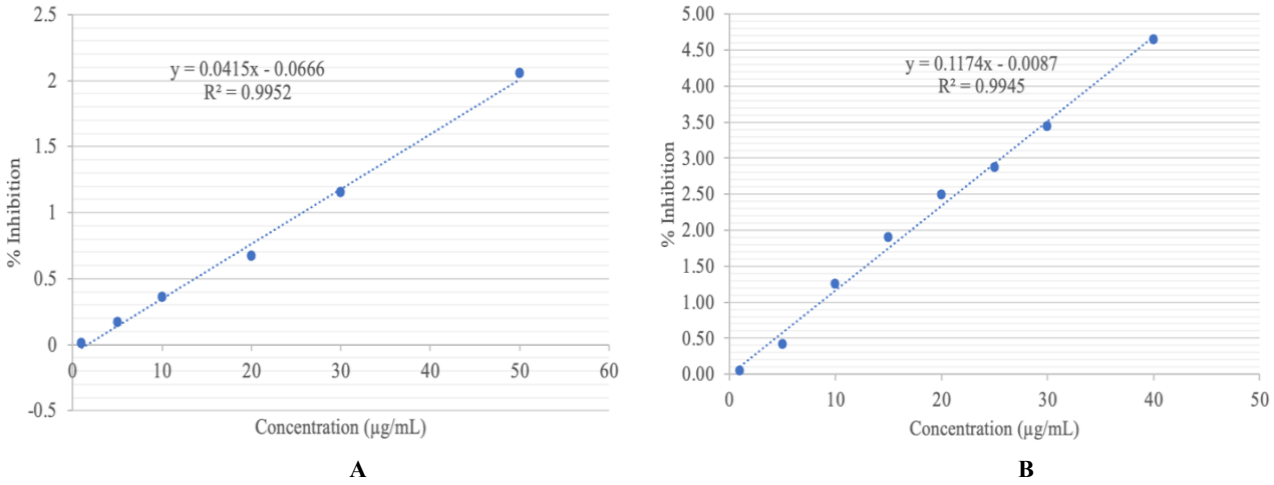

Figure 1. Calibration curves of: A. Gallic acid standard for Total Phenolic Content (TPC), expressed as mg gallic acid equivalents per g of dry extract, and B. Quercetin standard for Total Flavonoid Content (TFC), expressed as mg quercetin equivalents per g of dry extract

Total Flavonoid Content (TFC) was determined using the aluminum chloride colorimetric assay as described by Chokchaisiri et al. (2025), with minor adaptations for microplate analysis. Extract-stock solutions (1 mg/mL) were diluted to the same working concentration range (1-50 ppm) used for TPC determination to maintain consistency across assays. In a 96-well microplate, 100 µL of diluted extract or standard solution was mixed with 100 µL of 2% (w/v) aluminum chloride solution. The mixtures were incubated at room temperature for 20 min to allow complex formation, after which absorbance was measured at 415 nm. Quercetin was used as the reference standard. A calibration curve was prepared in the concentration range of 5-50 µg/mL, yielding linear regression with $R^2 > 0.999$ (Figure 1.B). A reagent blank containing methanol instead of sample was included for background subtraction. All standards and samples were measured in triplicate. TFC was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

Antioxidant activity assays

The antioxidant activity of cannabis extracts was evaluated using three widely applied in vitro assays: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay, and the Ferric Reducing Antioxidant Power (FRAP) assay. All reagents were of analytical grade. The DPPH and ABTS assays were performed following the protocols described by Ilyasov et al. (2020), while the FRAP assay was conducted according to the method applied for *Cannabis sativa* extracts by Judžentienė et al. (2023). No procedural deviations from the cited methods were introduced.

For the DPPH and ABTS assays, extract solutions were tested over a series of concentrations (six to eight concentrations within the range of 1-500 µg/mL, depending on assay sensitivity) to generate dose-response curves. Percentage inhibition was calculated relative to solvent blanks. Trolox was used as a positive control/reference antioxidant and was analyzed over the same concentration ranges to verify assay performance. Dose-response curves (% inhibition vs. concentration) were fitted using nonlinear regression (four-parameter logistic model), and half-maximal inhibitory concentrations (IC_{50}) were calculated using GraphPad Prism (version X; GraphPad Software, USA). Goodness-of-fit was evaluated by visual inspection of residuals and coefficients of determination (R^2). Each concentration was measured in triplicate.

For the FRAP assay, antioxidant capacity was determined based on the reduction of the Fe^{3+}-TPTZ complex to Fe^{2+} under acidic conditions, following the referenced protocol. Absorbance was measured at the specified wavelength after incubation, and FRAP values were calculated from a Ferrous Sulfate ($FeSO_4$) calibration curve. Results were expressed as millimoles of ferrous ion equivalents per 100 g of dry extract (mmol Fe^{2+}/100 g).

Trolox was included as a reference antioxidant to confirm assay performance. All assays were conducted independently for each biological replicate, with three technical replicates per concentration. Data are presented as mean±standard deviation prior to statistical analysis.

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 26 (IBM Corp., USA). Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. When data met the assumptions of normality and homoscedasticity, differences among cultivars within each assay (TPC, TFC, DPPH, ABTS, and FRAP) were analyzed by one-way Analysis of Variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test. For pairwise comparisons involving the reference standard (Trolox) and individual cultivar extracts, Student's t-test was applied when assumptions were met. When data did not satisfy assumptions of normality or equal variance, non-parametric alternatives (Kruskal-Wallis test followed by Dunn's post hoc test) were used as appropriate. Statistical significance was accepted at $p<0.05$. All results are presented as mean±standard deviation ($n = 3$).

RESULTS AND DISCUSSION

Extraction yield

The extraction yields of the four *Cannabis sativa* cultivars ranged from 31.56% to 52.76% (w/w, dry weight basis, Table 2), with Top Cherry Gas showing the highest yield (52.76%) and Wedding Cake the lowest (31.56%), reflecting cultivar-dependent differences in the abundance of ethanol-soluble metabolites (Andre et al. 2016, Aizpurua-Olaizola et al. 2016). When examined in relation to chemical composition, extraction yield showed a closer association with total cannabinoid content than with TPC or TFC. For example, Dur Burger exhibited a relatively high yield despite low TPC/TFC but contained high CBD levels, indicating that non-phenolic constituents, particularly cannabinoids, contribute substantially to the overall extract mass due to their lipophilic nature and high ethanol solubility (Hazekamp et al. 2004). These findings demonstrate that extraction yield alone cannot be used as a proxy for specific bioactive classes, but rather reflects the integrated phytochemical composition of each chemotype (Azmir et al. 2013). From an applied perspective, cultivars with higher yields may offer processing advantages; however, biological functionality depends primarily on the type and proportion of key compounds. Therefore, cultivar selection for product development should be based on an integrated consideration of extraction yield, cannabinoid profiles, and TPC/TFC.

Cannabinoid chemotypes of four cultivars

HPLC analysis of ten cannabinoids revealed clear compositional differences among the cultivars, allowing classification into two major chemotypic groups: CBD-dominant and THC-dominant (Table 3). The cultivar Dur Burger was categorized as CBD-dominant, with a high total CBD content (569.38±0.27 mg/g dry extract), primarily comprising CBD, CBDA, and CBDV, and comparatively low Δ^9-THC (21.40±0.01 mg/g dry extract). Such a profile is consistent with cultivars commonly used in medical contexts because of their non-intoxicating nature. Nevertheless, it should be emphasized that the present study focused exclusively on phytochemical composition and in vitro antioxidant activity and did not evaluate pharmacological efficacy or clinical safety (Iffland and Grotenhermen 2017).

In contrast, Sensi Dawg, Top Cherry Gas, and Wedding Cake exhibited THC-dominant chemotypes, with Sensi Dawg showing the highest total THC content (656.81±0.34 mg/g dry extract). This pattern is consistent with contemporary commercial breeding strategies that prioritize psychoactive potency and market demand (Hazekamp and Fischedick 2012; ElSohly et al. 2016). Examination of minor cannabinoids revealed pronounced cultivar-dependent variation: Top Cherry Gas and Wedding Cake contained higher levels of CBG, whereas Dur Burger was enriched in CBC. These differences reflect variability in biosynthetic flux within the cannabinoid pathway, where multiple synthase enzymes compete for the common precursor cannabigerolic acid (CBGA) to produce CBD(A), THC(A), CBC(A), or CBG (Flores-Sanchez and Verpoorte 2008). Such variation in minor cannabinoids is biologically relevant and may contribute to functional properties through potential synergistic interactions, often referred to as the entourage effect (Russo 2011).

Table 2. Extraction yield of four *Cannabis sativa* cultivars obtained by ethanol extraction (dry weight basis)

Cultivar	Initial dry weight (g)	Extract weight (g)	Yield (%)
Sensi Dawg	50.00	17.69	35.38
Top Cherry Gas	50.00	26.38	52.76
Wedding Cake	50.00	15.78	31.56
Dur Burger	50.00	20.34	40.68

Table 3. Content of 10 cannabinoids (mg/g dry extract) in four *Cannabis sativa* cultivars by HPLC

Cannabinoid	Cultivar			
	Sensi Dawg	Top Cherry Gas	Wedding Cake	Dur Burger
CBDV	ND	ND	ND	12.36 ± 0.01^{e}
CBDA	ND	ND	ND	$15.18\pm0.01^{d,e}$
CBGA	6.19 ± 0.00^{c}	6.62 ± 0.00^{d}	8.96 ± 0.00^{e}	ND
CBG	7.00 ± 0.00^{c}	26.58 ± 0.00^{c}	24.99 ± 0.01^{d}	17.68 ± 0.01^{d}
CBD	ND	ND	ND	556.07 ± 0.26^{b}
THCV	6.58 ± 0.00^{c}	5.08 ± 0.00^{d}	$6.63\pm0.00^{e,f}$	ND
CBN	50.70 ± 0.03^{b}	49.96 ± 0.02^{b}	51.09 ± 0.02^{c}	3.68 ± 0.00^{f}
Δ^9-THC	656.81 ± 0.34^{a}	590.04 ± 0.20^{a}	560.26 ± 0.22^{b}	21.40 ± 0.01^{c}
CBC	9.87 ± 0.00^{c}	6.14 ± 0.00^{d}	5.67 ± 0.00^{f}	$18.24\pm0.00^{c,d}$
THCA	ND	ND	9.31 ± 0.01^{e}	ND
Total THC	656.81 ± 0.34^{a}	590.04 ± 0.20^{a}	568.43 ± 0.23^{a}	21.40 ± 0.01^{c}
Total CBD	ND	ND	ND	569.38 ± 0.27^{a}

Note: ND indicates values below the limit of detection. Different superscript letters (a-f) within the same row indicate statistically significant differences among cultivars (One-Way ANOVA followed by Tukey's HSD test, $p<0.05$)

Relatively high levels of CBN (~50 mg/g dry extract) were detected in the THC-dominant cultivars. Previous studies have associated CBN accumulation with THC degradation under oxidative conditions or prolonged storage (Aizpurua-Olaizola et al. 2016). However, because this study did not systematically evaluate storage parameters (e.g., light exposure, temperature, or duration) and cannot exclude cultivar-specific genetic contributions to CBN levels, this interpretation should be regarded as tentative. Accordingly, the observed CBN abundance is best considered consistent with trends reported for high-THC cultivars rather than as direct evidence of THC oxidation, and this hypothesis cannot be explicitly tested within the current dataset. Overall, the chemotypic patterns observed here are consistent with previous reports on Thai-grown cannabis that documented substantial chemical diversity under local cultivation conditions (Tipparat et al. 2012). The present study extends this knowledge by integrating quantitative chemotype classification with antioxidant assessment under controlled cultivation, enabling more reliable cultivar-to-cultivar comparisons and functional interpretation.

Phenolic and flavonoid contents

Sensi Dawg and Top Cherry Gas exhibited the highest Total Flavonoid Content (TFC) and Total Phenolic Content (TPC), whereas Wedding Cake and Dur Burger showed markedly lower values (Table 4). Given the established role of phenolics and flavonoids as hydrogen and electron donors, these compositional differences are consistent with their stronger antioxidant performance in assays based on Hydrogen Atom Transfer (HAT) and reducing mechanisms (Gupta 2015; Bonini et al. 2018), as discussed below.

Variation in TPC and TFC among cultivars likely reflects underlying genetic background and differential allocation of metabolic flux among secondary metabolite pathways. Previous studies have proposed that chemotypes prioritizing cannabinoid biosynthesis may divert precursors from the phenylpropanoid pathway, resulting in reduced phenolic and flavonoid levels (Andre et al. 2016; Lewis et al. 2018). Although this hypothesis provides a plausible framework, the present dataset does not directly test such metabolic trade-offs, therefore, the observed patterns should be interpreted as associative rather than mechanistic.

Antioxidant activities (DPPH, ABTS, and FRAP)

The three antioxidant assays yielded distinct activity patterns, reflecting their different mechanistic bases. In the DPPH assay, which primarily reflects HAT mechanisms, Sensi Dawg and Top Cherry Gas exhibited the strongest activities, consistent with their high TPC and TFC values. In contrast, Wedding Cake and Dur Burger showed weaker DPPH responses, underscoring the dominant contribution of phenolic and flavonoid compounds in this assay. By contrast, the ABTS assay produced a different ranking: Dur Burger displayed the lowest IC_{50} (6.59±0.06 µg/mL) despite its relatively low TPC and TFC. Because ABTS accommodates both HAT and Single-Electron Transfer (SET) mechanisms and is responsive to compounds across a broader polarity range (Ilyasov et al. 2020), this result cannot be explained by phenolics alone. Previous studies have demonstrated that Cannabidiol (CBD) and certain minor cannabinoids can participate in SET-dominated antioxidant processes (Atalay et al. 2019; Pagano et al. 2023). Accordingly, it is plausible that CBD contributed to the ABTS activity of Dur Burger. However, this interpretation remains hypothetical, as isolated cannabinoids were not tested in the present study.

For the FRAP assay, which reflects reducing capacity, only modest numerical differences were observed among the four cultivars, and no statistically significant differences were detected ($p>0.05$), whereas Trolox showed significantly higher activity (Table 5). This limited differentiation suggests that FRAP may be less sensitive to chemotypic variation under the present experimental conditions. In particular, FRAP primarily measures the overall electron-donating ability toward the Fe^{3+}-TPTZ complex in an acidic environment, a response that can be contributed by multiple redox-active constituents (including non-phenolic compounds) and may therefore mask differences attributable to specific cannabinoid or phenolic profiles. Consequently, variations in chemotype that are clearly resolved by radical-scavenging assays (DPPH, ABTS) may be compressed in FRAP measurements. Standard deviation values were small relative to means across assays, indicating good repeatability. This consistency reflects controlled cultivation conditions, pooling of multiple plants within each biological replicate, and standardized analytical protocols, thereby enhancing confidence in the observed cultivar-dependent trends.

Table 4. Total phenolic and flavonoid contents of four *Cannabis sativa* cultivar extracts

Cultivar	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Sensi Dawg	69.29±5.4^{a}	65.53±0.69^{b}
Top Cherry Gas	69.24±2.79^{a}	70.67±0.85^{a}
Wedding Cake	13.53±0.78^{b}	38.14±1.96^{c}
Dur Burger	21.61±1.44^{b}	26.04±2.02^{d}

Note: Values are expressed as mean±standard deviation (n = 3). Different superscript letters (a-d) within the same column indicate statistically significant differences among cultivars (one-way ANOVA followed by Tukey's HSD test, $p<0.05$)

Table 5. Antioxidant activities of four *Cannabis sativa* cultivar extracts

Cultivar	DPPH (IC_{50}, µg/mL)	ABTS (IC_{50}, µg/mL)	FRAP (mmol Fe^{2+}/100 g extract)
Sensi Dawg	74.6±1.08^{b}	9.1±0.04^{d}	21.26±0.20^{b}
Top Cherry Gas	76.86±0.96^{b}	8.39±0.10^{c}	19.70±1.58^{b}
Wedding Cake	320.42±3.56^{c}	9.10±0.17^{d}	17.23±1.02^{b}
Dur Burger	>500^{d}	6.59±0.06^{b}	15.61±1.38^{b}
Trolox	8.06±0.19^{a}	5.90±0.00^{a}	2427.50±28.26^{a}

Note: Values are expressed as mean±standard deviation (n = 3). Different superscript letters (a-d) within the same column indicate statistically significant differences among cultivars (one-way ANOVA followed by Tukey's HSD test, $p<0.05$). Trolox was included as a reference antioxidant

Although classical biodiversity indices were not assessed, the chemotypic and secondary metabolite diversity observed in this study represents an important dimension of agro-biodiversity and genetic resource diversity in *C. sativa*. Maintaining multiple chemotypes (e.g., CBD-dominant, THC-dominant, phenolic-rich) supports diverse pharmaceutical and nutraceutical applications while promoting germplasm conservation and sustainable bioresource management in Thailand. Integrating chemotype data with functional performance thus provides a practical framework for cultivar selection that enhances value addition without compromising genetic diversity.

Limitations

This study has several limitations: (i) only four cultivars from a single licensed indoor facility and a single harvest season were examined, which limits the generalizability of the results across broader genetic backgrounds, cultivation systems, and environmental conditions; (ii) A single extraction solvent (ethanol) was used, which may introduce extraction bias and restrict the recovery of compound classes with different polarities, thereby influencing the observed cannabinoid, phenolic, and flavonoid profiles; (iii) antioxidant activity was assessed exclusively using in vitro assays meaning that the results reflect chemical reactivity rather than biological efficacy in cellular or in vivo systems, and thus cannot be directly extrapolated to physiological relevance; and (iv) isolated cannabinoids were not tested, which constrains mechanistic interpretation and prevents definitive attribution of specific antioxidant effects to individual compounds such as CBD or minor cannabinoids. Future studies should address these limitations by including a larger number of cultivars across multiple sites and seasons, applying complementary extraction systems, performing formal correlation and multivariate analyses, and validating functional outcomes in biological models. Such approaches will strengthen causal inference, enhance external validity, and improve the applicability of chemotypic profiling for sustainable utilization and product development.

In conclusion, this study demonstrates pronounced chemotypic and antioxidant diversity among the four *C. sativa* cultivars cultivated in Thailand, highlighting the value of chemotype-based evaluation for functional applications. Distinct profiles were evident between THC-dominant cultivars, phenolic-rich cultivars and a CBD-dominant cultivar, indicating that antioxidant behavior in cannabis reflects contributions from multiple metabolite classes rather than phenolics alone. Notably, the CBD-dominant cultivar Dur Burger exhibited the strongest ABTS activity (IC_{50} = 6.59±0.06 µg/mL), despite comparatively low TPC/TFC, highlighting a chemotype-specific antioxidant signature. Collectively, these findings emphasize that cultivar identity is a critical determinant of chemical composition and functional potential. Beyond product-oriented relevance, the observed chemotypic diversity represents an important dimension of agro-biodiversity and genetic resource variation in *C. sativa*, supporting germplasm conservation and sustainable utilization of cannabis as a bioresource in Thailand. By integrating phytochemical profiling with functional assessment, this work provides a concise reference framework for targeted cultivar selection while preserving chemical and genetic diversity. Future research should build directly on the present limitations by expanding the number of cultivars across multiple cultivation sites and seasons, applying complementary extraction systems, and testing isolated cannabinoids to clarify mechanistic contributions. Validation in biological models will further strengthen causal inference and enhance the translational relevance of chemotype-guided cannabis utilization.

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