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Comparative Studies of Antioxidant Activities of Herbal Formulation of *Curcuma longa* L. and *Cannabis sativa* L. Plant Extracts: A Phytochemical Approach

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The present study investigates the comparative antioxidant potential of *Curcuma longa* L. and *Cannabis sativa* L. extracts, individually and in combination, with the aim of developing novel herbal formulations. Multiple extraction techniques were employed for *C. longa*, including aqueous, heated, ethanol and Soxhlet methods, while *C. sativa* extracts were obtained *via* hydro-distillation of essential oil and cold pressing of hemp seed oil. Phytochemical screening confirmed the presence of phenolics, flavonoids, alkaloids, curcuminoids, terpenoids, cannabinoids and fatty acids, which are known contributors to antioxidant and antimicrobial activities. Characterization using UV-vis, TLC, HPLC, FTIR and GC-MS validated the presence of key functional groups and bioactive metabolites. Antioxidant capacity was determined using the DPPH radical scavenging assay, with ascorbic acid serving as the standard (IC_{50} = 12.68 µg/mL). Results indicated that *C. longa* ethanolic extract exhibited strong radical scavenging activity (IC_{50} = 21.01 µg/mL; 85.2% inhibition at 320 µg/mL), whereas *C. sativa* hemp seed oil showed weak activity (maximum 10.01% inhibition at 320 µg/mL; IC_{50} > 320 µg/mL) and essential oil demonstrated moderate potential. Importantly, the combined formulation of *C. longa* ethanolic extract with *C. sativa* essential oil displayed significantly enhanced antioxidant capacity (up to 85.2% scavenging at 100 µg/mL with an IC_{50} of 45.6 µg/mL), suggesting synergistic interactions among phytoconstituents. GC-MS profiling further revealed that *C. longa* ethanolic extract was rich in dehydrozingerone (35.82%), turmerones (18.29%) and curlone (8.60%), whereas hemp seed oil was dominated by linoleic acid (56.11%), α-linolenic acid (17.02%) and oleic acid (13.64%), reflecting a complementary balance of phenolic antioxidants and polyunsaturated fatty acids. These findings emphasize the chemical and biological complementarity of turmeric and cannabis, supporting their integration in polyherbal formulations aimed at mitigating oxidative stress. The study highlights the potential of turmeric–cannabis combinations as natural alternatives for antioxidant therapy, providing a basis for further pharmacological and nutraceutical applications.

Keywords: *Curcuma longa, Cannabis sativa*, Phytochemical analysis, Antioxidant activity, Herbal formulation.

INTRODUCTION

The exploration of plant-derived bioactive compounds has gained considerable attention in recent decades owing to their structural diversity, wide range of biological activities and potential applications in drug discovery and functional formulations [1,2]. Oxidative stress, a biochemical state arising from the excessive generation of reactive oxygen species (ROS), is strongly linked to pathological processes including cancer, inflammation, cardiovascular disorders and neurodegenerative diseases [3]. At the molecular level, ROS initiate lipid peroxidation, protein oxidation and DNA damage by interacting with double bonds, sulfhydryl groups and aromatic residues, thereby disturbing the cellular redox balance. In this context, antioxidants play a critical role as electron or hydrogen atom donors, stabilizing free radicals and halting oxidative chain reactions [4]. While synthetic antioxidants such as butylated hydroxytoluene (BHT) have been used, their safety concerns highlight the importance of natural compounds from medicinal plants that exhibit multiple mechanisms of antioxidant action, including radical scavenging, metal chelation and modulation of redox-sensitive signaling pathways [5,6].

Among the most prominent medicinal plants, *Curcuma longa* L. (turmeric) has been extensively studied for its rich chemical profile. The principal active component, curcumin, is a diferuloylmethane polyphenol characterized by two methoxyphenol rings connected *via* a conjugated heptadienone chain containing diketone functionality [7]. This conjugated system

allows for resonance stabilization of free radicals and contributes to curcumin's strong radical scavenging potential [8]. Beyond curcumin, turmeric rhizomes contain demethoxycurcumin and bisdemethoxycurcumin (which differ in the number of methoxy groups on the aromatic rings), along with other diarylheptanoids and further classes of phytochemicals such as flavonoids, tannins, alkaloids and terpenoids [9,10]. *In vitro* and *in vivo* studies have reported that curcumin and its derivatives neutralize hydroxyl, superoxide and nitric oxide radicals, while also downregulating lipid peroxidation processes. From a chemical perspective, the phenolic –OH and conjugated C=C–C=O moieties are responsible for hydrogen donation and electron transfer, respectively, which are the fundamental mechanisms of antioxidant activity [11,12].

Despite these well-established chemical properties, curcumin suffers from poor aqueous solubility and low oral bioavailability due to extensive metabolism and rapid systemic elimination [13]. To overcome these limitations, considerable research has focused on nanostructured delivery systems, liposomal encapsulation, self-emulsifying formulations and polymer conjugates that improve solubility, stability and targeted delivery of curcumin [14]. These advancements highlight the importance of chemical modification and formulation science in enhancing the clinical applications of curcumin.

On the other hand, *Cannabis sativa* L. is another chemically complex plant producing bioactive compounds including cannabinoids, terpenoids, flavonoids and fatty acids [15]. The major cannabinoids such as cannabidiol and Δ^9-tetrahydrocannabinol, belong to a class of terpenophenolic compounds formed *via* the polyketide and isoprenoid pathways [16]. The terpenes (*e.g.* β-caryophyllene, limonene, myrcene) present in cannabis essential oils add to the antioxidant and antimicrobial properties [17]. Furthermore, hemp seeds of *C. sativa* are rich in polyunsaturated fatty acids, particularly omega-6 (linoleic acid) and omega-3, which play essential roles in maintaining cell membrane stability, reducing oxidative stress and preventing lipid peroxidation [18].

A promising aspect lies in the synergistic combination of *C. longa* and *C. sativa* extracts, which integrates chemically diverse phytoconstituents to provide complementary mechanisms. Curcumin, with its phenolic and diketone functionalities, directly scavenges radicals, while cannabis-derived fatty acids and terpenoids stabilize biomembranes and enhance anti-inflammatory responses [19]. Furthermore, the lipid matrix from hemp seed oil can act as a natural solubilizer, potentially improving the dissolution and absorption of curcumin [20]. Such chemical synergy aligns with the traditional polyherbal practices in Ayurveda and Chinese medicines, which often employ multi-component systems to balance efficacy and reduce toxicity [21,22].

Despite a growing body of literature, significant gaps remain, as most studies have examined turmeric and cannabis separately, with limited focus on their comparative phytochemistry or formulation-based synergistic potential. Furthermore, variability in extraction methods, plant cultivars and standardization practices often lead to inconsistent results [23,24]. To address these gaps, systematic comparative studies using controlled extraction, chemical characterization and validated antioxidant assays are necessary.

This study aims to elucidate the antioxidant potential of *C. longa* and *C. sativa*, both individually and in combination, providing a rational basis for developing potent natural antioxidant formulations. By integrating traditional ethnomedicinal knowledge with modern scientific approaches, the work seeks to advance phytopharmacological research, promote innovative plant-based nutraceutical strategies and offer promising solutions for mitigating oxidative stress and associated health disorders.

EXPERIMENTAL

The collection process for *Curcuma longa* L. involved procuring fresh rhizomes from the National Agricultural University, Pantnagar, India. Multiple varieties of *C. longa* were selected to ensure a diverse range of bioactive compounds for the study. The varieties *C. longa* TCP 129, NYST-11, RH7/90, NYST-24 and Sughandham were selected for their known medicinal properties and previous research suggesting their potential efficacy. The rhizomes were freshly harvested to maintain the integrity of their phytoconstituents, which are crucial for subsequent extraction and analysis processes. The *Cannabis sativa* L. samples were collected Pant nagar, India, known for its conducive climate and soil conditions favourable for the growth of medicinal plants. The collection was carried out at the end of August 2024 a time when the plant reached its peak in terms of essential oil content. The plant material included whole plants and seeds, which were carefully harvested and then authenticated by Botanical Survey of India, Dehradun.

Preparation of extracts: The bioactive compounds from *C. longa* and *C. sativa* were extracted using specialized techniques to ensure high yield and purity.

***Curcuma longa* extraction:** The rhizomes of *C. longa* were extracted using ethanol by combining maceration and Soxhlet techniques to obtain a pooled methanolic extract. In the maceration method, freshly homogenized rhizomes were soaked in 95% ethanol for seven days with corrected shaking, followed by filtration and concentration under reduced pressure. In parallel, dried and powdered rhizomes were subjected to Soxhlet extraction with ethanol for 5 h and the extract was similarly concentrated. Both extracts were pooled to yield a single ethanolic extract, which was collected in sterile vials and stored at 4 ºC until further use in phytochemical and antioxidant assessment.

***Cannabis sativa* extraction:** Hemp seed oil from *C. sativa* L. was obtained using the cold-pressing method. Cleaned and dried seeds were subjected to mechanical pressing at low temperatures to prevent degradation of heat-sensitive bioactive compounds. The extracted oil was filtered to remove particulate matter and stored in Amber-coloured glass bottles at 4 ºC until further use in phytochemical and antioxidant evaluations.

Phytochemical analysis: Phytochemical screening of *C. longa* and *C. sativa* extracts was performed using standard qualitative tests to detect major classes of bioactive compounds [25]. Alkaloids were identified using Wagner's reagent, while carbohydrates were confirmed by Molisch's test. Cardiac glycosides were detected through Keller Kelliani's test, flavonoids by the alkaline reagent test, phenols by the ferric chloride

test and phlobatannins by the hydrochloric acid precipitate test. The presence of amino acids and proteins was determined using the ninhydrin test, saponins by the foam test, steroids by the Liebermann-Burchard test, tannins by Braymer's test and terpenoids by Salkowki's test. Quinones and coumarins were confirmed by conc. HCl and NaOH/HCl reactions, respec-tively. Furthermore, curcumin was isolated from turmeric extracts through solvent partitioning with benzene and NaOH, followed by acid precipitation, vacuum filtration and drying and the purified curcumin was used for further evaluation.

Development of herbal formulations: To develop a synergistic herbal formulation, *C. longa* ethanolic extract (containing curcumin) and *C. sativa* hemp seed oil (rich in omega fatty acids) were utilized as the primary ingredients. The formulations were prepared in varying ratios of *C. longa* to hemp oil, including 100:0, 80:20, 50:50, 20:80 and 0:100, to evaluate their combined effects. By combining these two potent natural compounds, the formulation aimed to harness the therapeutic benefits of curcumin and omega fatty acids synergistically.

Evaluation of antioxidant activity by DPPH radical scavenging assay: The antioxidant activity of *C. longa* extracts, *C. sativa* seed oil and their combination was assessed using the DPPH radical scavenging assay. A 0.004% DPPH solution in ethanol was prepared and mixed with different concentrations of extracts (20, 60, 80 and 120 mg/mL), followed by incubation in dark at room temperature for 30 min. Ascorbic acid was used as the standard and ethanol as blank. Absorbance was measured at 517 nm and percentage inhibition was calculated to determine IC_{50} values. The antioxidant activity index (AAI) was calculated according to El *et al.* [26], with activity classified as poor (AAI < 0.05), moderate (0.05-1.0), or strong (AAI > 1.0). For *C. sativa*, the same procedure was applied with minor modifications [27]. A combined formulation of *C. longa* ethanolic extract and *C. sativa* essential oil was also evaluated following Yekefallah & Raofie [28]. All assays were conducted in triplicate for accuracy.

Gas chromatography-mass spectrometry (GC-MS) analysis: The pooled ethanolic extract of *C. longa* rhizomes and the cold-pressed hemp seed oil of *C. sativa* were subjected to GC-MS analysis to identify volatile and semi-volatile phytoconstituents. The analysis was carried out using a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (FID) and an autosampler. Separation was achieved on a fused silica capillary column (OV-1, DB-1, 30 m × 0.53 mm, 0.5 μm film thickness) under a programmed oven temperature starting at 75 ºC with a 1-min hold, followed by an increase at 5 ºC/min to 240 ºC with a final hold of 5 min. Helium served as the carrier gas at a constant flow rate of 1.0 mL/min. The transmission line was maintained at 300 ºC, while the ion source for electron impact (EI) was set at 220 ºC with an electron energy of 70 eV. The total GC run time was 39 min.

For sample preparation, 50 μL of each extract was diluted in 500 μL of ethyl acetate, vortexed and transferred into GC vials. A 1 μL aliquot was injected into the GC-MS system in split mode. The volatile constituents were identified by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) library. The relative abundance of each compound was expressed as a percentage of the total peak area from the chromategram [29].

Ethical considerations: The study adhered to ethical guidelines for plant material collection, authorized by National Agricultural University, Pantnagar and the Botanical Survey of India, Dehradun. Laboratory experiments complied with safety protocols and hazardous materials were disposed of responsibly. Proper authentication ensured data transparency and integrity in reporting findings.

RESULTS AND DISCUSSION

Phytochemical analysis: The phytochemical results of *C. longa* L. and *C. sativa* L. extracts in Table-1 revealed distinct variations in their bioactive compound composition, with *C. longa* exhibiting a strong presence of alkaloids, flavonoids, phenols, steroids, terpenoids and curcumin, while *C. sativa* showed a strong presence of phenols, steroids, quinones and coumarins. The exclusive presence of curcumin in *C. longa* underscores its well-known antioxidant and anti-inflammatory properties, whereas the higher concentration of quinones and coumarins in *C. sativa* suggests a distinctive antimicrobial mechanism. Furthermore, *C. longa* demonstrated a higher content of carbohydrates, cardiac glycosides, proteins, amino acids, phlobatannins, saponins and tannins, indicating a broader spectrum of bioactive compounds that contribute to its medicinal value.

TABLE-1
PHYTOCHEMICAL RESULTS OF
Curcuma longa L. AND *Cannabis sativa* EXTRACT

Test/compound	*Curcuma longa* L.	*Cannabis sativa* L.
Alkaloids (Wagner's test)	+++	++
Carbohydrates (Molisch's test)	++	+
Cardiac glycosides (Keller-Killiani test)	++	++
Flavonoids (alkaline reagent test)	+++	++
Phenols (ferric chloride test)	+++	+++
Phlobatannins (precipitate test)	++	+
Amino acids & proteins (ninhydrin test)	++	+
Saponins (foam test)	++	+
Steroids (Liebermann-Burchard test)	+++	+++
Tannins (Braymer's test)	++	+
Terpenoids (Salkowki's test)	+++	++
Quinones	++	+++
Coumarins	+	+++
Curcumin (specific for *C. longa*)	+++	–

+++ = Strongly present; ++ = Moderately present; + = Slightly present; – = Absent

These findings suggest that *C. longa* may have stronger antioxidant potential due to the high levels of flavonoids, phenols and terpenoids, which are known for their radical scavenging properties, while *C. sativa*, with its higher quinone and coumarin content, may exhibit enhanced antimicrobial activity. While both plant extracts contain significant bioactive compounds, their differing phytochemical compositions indicate that *C. longa* might be more effective as an antioxidant, whereas *C. sativa* could offer unique antimicrobial potential, providing a scientific basis for their potential therapeutic applications in herbal formulations as earlier reported [30,31].

Recent studies highlight that phenolics and flavonoids in turmeric play a key role in free radical scavenging through hydrogen donation and stabilization of reactive intermediates [32]. Curcuminoids have been reported to inhibit lipid peroxidation, enhancing their value in antioxidant formulations. Meanwhile, the coumarins and quinones identified in cannabis extracts have shown promise in antimicrobial strategies by interfering with bacterial redox cycling and biofilm formation [33]. This difference in phytochemical profiles aligns with reports that turmeric primarily contributes to antioxidant defense, while cannabis offers complementary antimicrobial mechanisms [34]. Importantly, the combined presence of multiple secondary metabolites broadens the pharmacological potential of both plants, supporting their inclusion in polyherbal formulations [35]. The contrasting yet complementary bioactive constituents underscore the rationale for developping turmeric–cannabis formulations, where antioxidant and antimicrobial properties can synergize to address oxidative stress and microbial infections simultaneously.

Antioxidant activity: The DPPH radical scavenging assay of standard ascorbic acid demonstrated a dose-dependent increase in antioxidant activity, as shown in Table-2. The absorbance at 517 nm (A_{517}) progressively decreased with increasing concentrations of ascorbic acid, confirming its strong free radical scavenging ability. At the lowest concentration of 5 μg/mL, the percentage inhibition of DPPH radicals was 17.855% with a standard deviation (S.D.) of 0.323, while at 10 μg/mL, the inhibition increased significantly to 50.098% (SD = 0.190). A further increase in concentration to 20 μg/mL resulted in 64.801% inhibition (SD = 0.137), while 40 μg/mL exhibited 78.242% inhibition (SD = 0.112). Near-total inhibition was observed at 80 μg/mL (95.033%, SD = 0.127) and 160 μg/mL (98.108%, SD = 0.116), with maximum inhibition recorded at 320 μg/mL, reaching 99.093% (SD = 0.180). The IC_{50} value, representing the concentration required to inhibit 50% of DPPH radicals, was determined as 12.68 μg/mL, indicating a high antioxidant potential of ascorbic acid. The low standard deviations across triplicates suggest consistent and reliable results. These findings confirm that ascorbic acid serves as a strong positive control in antioxidant activity assays, validating the experimental approach for assessing the antioxidant potential of herbal formulations.

The DPPH radical scavenging activity assay for standard curcumin exhibited a concentration-dependent increase in anti-oxidant activity, as shown in Table-3. The absorbance at 517 nm (A_{517}) progressively decreased with increasing curcumin concentration, indicating its ability to neutralize free radicals. At 5 μg/mL, the percentage inhibition was 16.357% (S.D. = 0.466), which was lower than that of ascorbic acid at the same concentration. As the concentration increased to 10 μg/mL, the inhibition rose to 32.243% (S.D. = 0.219), while at 20 μg/mL, a significant increase was observed, reaching 61.332% (S.D. = 0.090). The antioxidant activity continued to rise at 40 μg/mL, showing 70.240% inhibition (S.D. = 0.467) and at 80 μg/mL, it reached 76.705% (S.D. = 0.138). Near-complete inhibition was observed at 160 μg/mL (83.051%, SD = 0.153) and 320 μg/mL (85.219%, SD = 0.246), indicating curcumin's potent radical-scavenging properties. However, the IC_{50} value for curcumin was 21.01 μg/mL, which is higher than that of ascorbic acid (12.68 μg/mL), suggesting that curcumin requires a higher concentration to achieve 50%

TABLE-2
DPPH RADICAL SCAVENGING ACTIVITY RESULTS OF STANDARD ASCORBIC ACID

Controls		Experiment result $A_{517\ nm}$ 0.846	0.847	0.844	DPPH inhibition (%)			Mean	SD	IC_{50} (μg/mL)
Samples	Conc. (μg/mL)	Singlet	Duplicate	Triplicate	Singlet	Duplicate	Triplicate			
Std. ascorbic acid	5	0.695	0.693	0.696	17.849	18.182	17.536	17.855	0.323	12.68
	10	0.421	0.422	0.423	50.236	50.177	49.882	50.098	0.190	
	20	0.299	0.298	0.296	64.657	64.817	64.929	64.801	0.137	
	40	0.183	0.185	0.184	78.369	78.158	78.199	78.242	0.112	
	80	0.042	0.041	0.043	95.035	95.159	94.905	95.033	0.127	
	160	0.017	0.016	0.015	97.991	98.111	98.223	98.108	0.116	
	320	0.006	0.009	0.008	99.291	98.937	99.052	99.093	0.180	

TABLE-3
DPPH RADICAL SCAVENGING ACTIVITY RESULTS OF *Curcuma longa* L.

Controls		Experiment result $A_{517\ nm}$ 0.846	0.847	0.844	DPPH inhibition (%)			Mean	SD	IC_{50} (μg/mL)
Samples	Conc. (μg/mL)	Singlet	Duplicate	Triplicate	Singlet	Duplicate	Triplicate			
Std. ascorbic acid	5	0.709	0.704	0.709	16.194	16.883	15.995	16.357	0.466	21.01
	10	0.572	0.573	0.574	32.388	32.349	31.991	32.243	0.219	
	20	0.328	0.327	0.326	61.229	61.393	61.374	61.332	0.090	
	40	0.252	0.248	0.255	70.213	70.720	69.787	70.240	0.467	
	80	0.198	0.196	0.197	76.596	76.860	76.659	76.705	0.138	
	160	0.143	0.145	0.142	83.097	82.881	83.175	83.051	0.153	
	320	0.127	0.123	0.125	84.988	85.478	85.190	85.219	0.246	

TABLE-4
DPPH RADICAL SCAVENGING ACTIVITY RESULTS OF STANDARD HEMP SEED OIL

Controls		Experiment result $A_{517\ nm}$ 0.846	0.847	0.844	DPPH inhibition (%)			Mean	SD	IC_{50} (μg/mL)
Samples	Conc. (μg/mL)	Singlet	Duplicate	Triplicate	Singlet	Duplicate	Triplicate			
Standard ascorbic acid	5	0.836	0.836	0.836	1.1820	1.2987	0.9479	1.1429	0.1787	> 320
	10	0.827	0.825	0.824	2.2459	2.5974	2.3697	2.4043	0.1783	
	20	0.815	0.813	0.812	3.6643	4.0142	3.7915	3.8233	0.1771	
	40	0.802	0.801	0.800	5.2009	5.4309	5.2133	5.2817	0.1294	
	80	0.799	0.796	0.798	5.5556	6.0213	5.4502	5.6757	0.3039	
	160	0.784	0.782	0.785	7.3286	7.6741	6.9905	7.3311	0.3418	
	320	0.762	0.760	0.761	9.9291	10.2715	9.8341	10.0116	0.2301	

radical inhibition compared to ascorbic acid. While curcumin exhibits strong antioxidant activity, its potency is lower than that of ascorbic acid, highlighting differences in the radical-scavenging efficiency of different compounds. These results validate the antioxidant potential of curcumin, supporting its use in herbal formulations derived from *C. longa* L. for therapeutic applications.

The DPPH radical scavenging activity assay for hemp seed oil, as shown in Table-4, demonstrated significantly lower antioxidant activity compared to ascorbic acid and curcumin, with a concentration-dependent but minimal increase in radical inhibition. At 5 μg/mL, the percentage inhibition was only 1.1429% (S.D. = 0.1787) and even at the highest tested concentration of 320 μg/mL, the inhibition reached only 10.0116% (S.D. = 0.2301). Intermediate concentrations exhibited gradual increases in antioxidant activity, with 2.4043% inhibition at 10 μg/mL (S.D. = 0.1783), 3.8233% at 20 μg/mL (S.D. = 0.1771), 5.2817% at 40 μg/mL (S.D. = 0.1294) and 7.3311% at 160 μg/mL (S.D. = 0.3418). The IC_{50} value for hemp seed oil was determined to be >320 μg/mL, indicating that even at the highest tested concentration, it was unable to reach 50% DPPH inhibition. These results suggest that hemp seed oil possesses weak antioxidant potential compared to curcumin and ascorbic acid. While hemp seed oil contains beneficial fatty acids and bioactive compounds, its radical-scavenging activity is relatively low, implying that its therapeutic effects may be more relevant in anti-inflammatory or nutritional contexts rather than as a primary antioxidant source.

The comparative antioxidant activity of ascorbic acid, curcumin and hemp seed oil using the DPPH assay, as shown in Table-5; Fig 1), highlights significant differences in their radical scavenging potential. Ascorbic acid exhibited the highest antioxidant activity, with 17.855% inhibition at 5 μg/mL, reaching 50.098% at 10 μg/mL and achieving near-total inhibition (99.093%) at 320 μg/mL, with an IC_{50} value of 12.68 μg/mL, indicating its strong radical scavenging efficiency. Curcumin showed moderate antioxidant activity, starting at 16.357% inhibition at 5 μg/mL, increasing to 32.243% at 10 μg/mL and reaching 85.219% at 320 μg/mL. However, its IC_{50} value of 21.01 μg/mL suggests that curcumin requires a higher concentration than ascorbic acid to achieve 50% radical inhibition. In contrast, hemp seed oil exhibited significantly weaker antioxidant potential, with only 1.025% inhibition at 5 μg/mL, 2.404% at 10 μg/mL and 10.012% at 320 μg/mL, failing to reach IC_{50}

TABLE-5
COMPARATIVE ANTIOXIDANT ACTIVITY RESULTS OF ASCORBIC ACID, CURCUMIN AND HEMP SEED OIL USING THE DPPH ASSAY

Conc. (μg/mL)	Std. ascorbic acid	Curcumin	Hemp seed oil
0	0.000	0.000	0.000
5	17.855	16.357	1.025
10	50.098	32.243	2.404
20	64.801	61.332	3.823
40	78.242	70.240	5.282
80	95.033	76.705	5.676
160	98.108	83.051	7.331
320	99.093	85.219	10.012

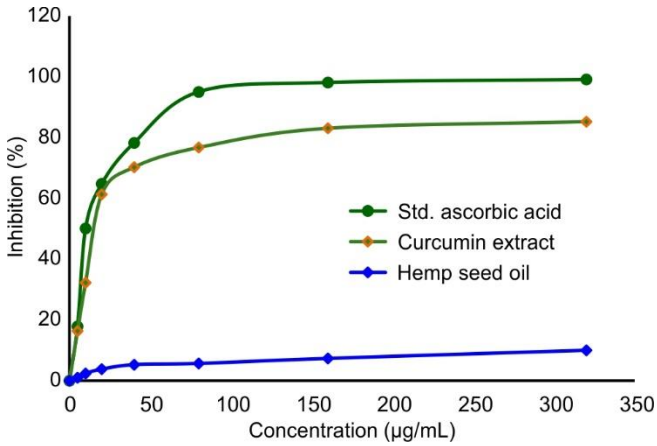

Fig. 1. Antioxidant activity of the tested extracts as determined by the DPPH radical scavenging assay

even at the highest tested concentration (> 320 μg/mL). These findings indicate that ascorbic acid is the most potent antioxidant, followed by curcumin powder with moderate activity, while hemp seed oil has minimal radical scavenging properties. The results suggest that formulations containing *C. longa* extracts may provide better antioxidant benefits than those derived from *C. sativa*, reinforcing their potential therapeutic applications in oxidative stress-related conditions.

Present findings are consistent with the prior literature. Curcumin's antioxidant activity *via* DPPH has been widely documented and a comparative study found that hydrogenated derivatives of curcumin (*e.g.* tetrahydrocurcumin) show stronger radical scavenging than curcumin itself and curcumin performs less strongly than standard antioxidants such as Trolox

under certain conditions [36]. Moreover, meta-analyses confirm curcumin's antioxidant potential *in vitro*, though often limited by solubility and stability issues in biological systems [37].

As for hemp seed oil, the weak DPPH activity in present work is also in line with several reports that pure oil or lipid-rich extracts generally show modest radical-scavenging in polar radical assays. For example, while hemp (*C. sativa* L.) seed oil holds nutritional value, its direct radical-scavenging activity in DPPH assays is relatively modest [38]. In a more recent report, methanolic extracts of hemp seed oils exhibited better antiradical activity (IC_{50} ~3.433 v/v in DPPH) than non-extracted oil, emphasizing that the phenolic/antioxidant compounds are more active when extracted into polar solvents rather than within intact lipid matrices [39]. This suggests that in present work, hemp seed oil in its non-polar form may not allow efficient interaction with the DPPH radical (which is in a polar solvent medium).

Moreover, composition of hemp seed oil is another key limiting factor. Hemp oil is rich in polyunsaturated fatty acids (PUFAs) like linoleic and α-linolenic acids (as confirmed from GC-MS), which are themselves more prone to oxidation than acting as radical scavengers. Some studies also point out that low levels of endogenous phenolics and tocopherols in oils limit direct radical-quenching capacity in DPPH-type assays [40]. Indeed, enhancing phenolic content (*e.g.* methanolic extraction or fortification) tends to increase DPPH scavenging [39]. From a comparative perspective, the present results indicate the following order in DPPH radical-scavenging activity: ascorbic acid > curcumin > hemp seed oil. This has implications for formulation: while curcumin (or curcuma-derived extracts) can provide meaningful antioxidant activity in phytopharmaceuticals, relying on unmodified hemp seed oil as a primary antioxidant source may not be sufficient. Nevertheless, hemp oil's health benefits may come from its fatty acid composition, anti-inflammatory effects and synergistic interactions when combined with phenolic-rich extracts, rather than acting as a potent radical scavenger alone. Present results align well with the literature: ascorbic acid remains a gold-standard radical scavenger, curcumin exhibits strong but context-dependent antioxidant action and hemp seed oil in its crude form is relatively weak in DPPH assays.

Herbal formulation of (*C. longa* L. + *C. sativa* L.)

Phytochemical analysis: The phytochemical screening of the combined herbal formulation containing *C. longa L.* and *C. sativa* L. revealed the presence of multiple bioactive compounds with varying concentrations. The formulation exhibited a strong presence of flavonoids, phenols, steroids and terpenoids, indicating a rich phytochemical profile with potential antioxidant, anti-inflammatory and antimicrobial properties. A moderate presence of alkaloids, carbohydrates, phlobatannins, cardiac glycosides, amino acids & proteins, saponins, tannins and quinones was also observed, suggesting a balanced composition of bioactive molecules (Table-6). Moreover, coumarins and curcumin were detected at a moderate level, confirming the contribution of *C. longa* to the formulation's therapeutic potential.

Antioxidant activity: The antioxidant activity of the combined *C. longa* and hemp seed oil extract was evaluated using the DPPH scavenging assay, as shown in Table-7. The results indicate a concentration-dependent increase in DPPH scavenging activity (%), demonstrating a direct correlation between extract concentration and antioxidant potential (Fig. 2). At 100 µg/mL, the extract exhibited the highest scavenging activity (85.2%), whereas at the lowest tested concentration (40 µg/mL), the scavenging activity was 65.7%. The IC_{50} values (the concentration required to inhibit 50% of free radicals) exhibited an inverse trend, decreasing from 60.9 µg/mL at 40 µg/mL concentration to 45.6 µg/mL at 100 µg/mL concentration, signifying an increase in antioxidant efficacy at higher concentrations. The reduction in IC_{50} values with increasing concentrations indicates enhanced radical-scavenging ability, suggesting that the combination of *C. longa* and hemp seed oil possesses strong antioxidant potential (Fig 3). These results highlight the synergistic effect of the formulation, making it a promising candidate for natural antioxidant applications in nutraceutical and pharmaceutical formulations.

TABLE-6
PHYTOCHEMICAL RESULTS OF COMBINE HERBAL FORMULATION

Test/compound	Herbal formulation (*Curcuma longa* L. + *Cannabis sativa* L.)
Alkaloids (Wagner's test)	++
Carbohydrates (Molisch's test)	++
Cardiac glycosides (Keller-Killiani test)	++
Flavonoids (alkaline reagent test)	+++
Phenols (ferric chloride test)	+++
Phlobatannins (precipitate test)	++
Amino acids & proteins (Ninhydrin test)	++
Saponins (foam test)	++
Steroids (Liebermann-Burchard test)	+++
Tannins (Braymer's test)	++
Terpenoids (Salkowki's test)	+++
Quinones	++
Coumarins	++
Curcumin (specific for *C. longa*)	++

TABLE-7
ANTIOXIDANT ACTIVITY DATA OF COMBINED *C. longa* AND HEMP SEED OIL EXTRACT USING DPPH ASSAY

Extract	Conc. (µg/mL)	DPPH scavenging activity (%)	IC_{50} (µg/mL)
C. longa + Hemp seed oil	100	85.2	45.6
C. longa + Hemp seed oil	90	82.5	48.2
C. longa + Hemp seed oil	80	78.9	50.9
C. longa + Hemp seed oil	70	75.6	53.1
C. longa + Hemp seed oil	60	72.1	55.7
C. longa + Hemp seed oil	50	69.3	58.2
C. longa + Hemp seed oil	40	65.7	60.9

GC analysis of ethanolic extract of *C. longa*: On the basis of comparison of the mass spectra with the NIST library, a total of 20 compounds were identified from the ethanolic extract of *C. longa* (Fig. 4; Table-8). Each compound was quantified based on its peak area percentage in the total ion chromatogram. Among the identified compounds, five major peaks were observed, corresponding to aR-turmerone (18.29%),

TABLE-8
LIST OF COMPOUNDS IDENTIFIED IN THE ETHANOLIC EXTRACT OF *Curcuma longa* BY GC-MS ANALYSIS

Peak	RT (min)	Area (%)	Height (%)	Compound name	m.f.
1	2.521	2.02	1.39	2-Pentanone, 5-hydroxy-	$C_5H_{10}O_2$
2	3.736	3.62	1.75	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$
3	7.707	3.14	2.14	Phenol, 2-methoxy-(guaiacol)	$C_7H_8O_2$
4	10.996	6.28	5.57	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$
5	13.362	0.52	0.66	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-(zingiberene)	$C_{15}H_{24}$
6	13.731	0.58	0.58	(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane	$C_{15}H_{24}$
7	14.686	0.54	0.44	Benzene, 1,4-dimethyl-2-(2-methylpropyl)-	$C_{12}H_{18}$
8	15.070	0.48	0.41	4,2,8-Ethanylylidene-2*H*-1-benzopyran, octahydro-2-methyl-	$C_{12}H_{18}O$
9	15.377	18.29	24.14	aR-Turmerone	$C_{15}H_{20}O$
10	15.437	8.46	10.65	Tumerone	$C_{15}H_{22}O$
11	15.812	8.60	11.45	Curlone	$C_{15}H_{22}O$
12	16.281	0.61	0.87	(6R,7R)-Bisabolone	$C_{15}H_{24}O$
13	16.523	1.18	1.17	Benzene, (1,1,4,6,6-pentamethylheptyl)-	$C_{18}H_{30}$
14	16.602	1.44	1.63	(E)-Atlantone	$C_{15}H_{22}O$
15	16.815	1.73	1.48	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-(isobornyl acrylate)	$C_{13}H_{20}O_2$
16	17.065	35.82	29.16	3-Buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-(dehydrozingerone)	$C_{11}H_{12}O_3$
17	17.767	1.36	0.80	6-(5-Hydroxy-4-methylidenecyclohex-2-en-1-yl)-2-methylhept-2-en-4-one	$C_{15}H_{22}O_2$
18	17.941	0.92	0.84	(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone	$C_{15}H_{22}O_2$
19	18.083	0.63	0.84	Hexadecanoic acid, methyl ester (methyl palmitate)	$C_{17}H_{34}O_2$
20	26.503	3.76	4.05	Ezlopitant, dehydro-	$C_{31}H_{36}N_2O$

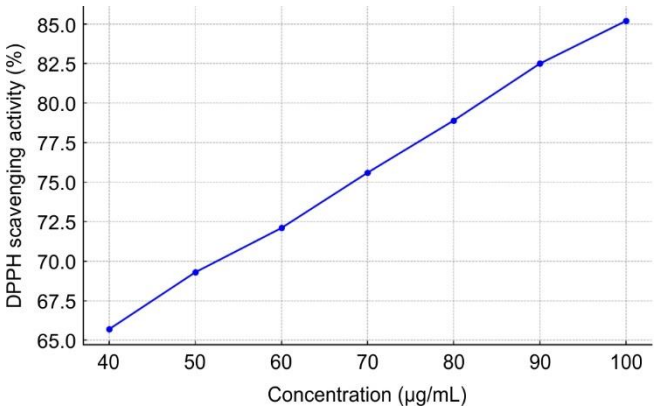

Fig. 2. DPPH radical scavenging activity of Herbal Formulation of *Curcuma longa* and hemp seed oil extracts at different concentrations

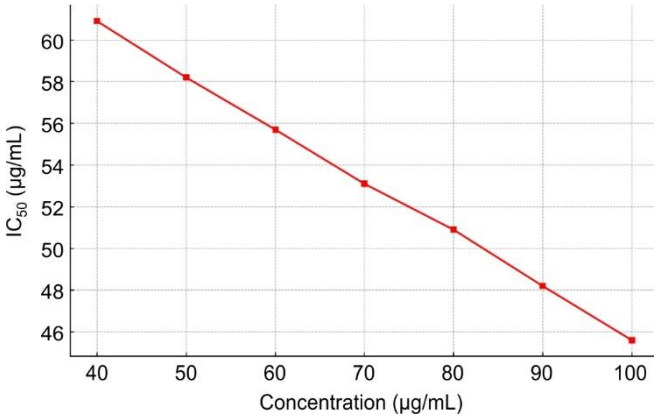

Fig. 3. Comparative IC_{50} values of Herbal Formulation of *Curcuma longa* and hemp seed oil extracts indicating their relative antioxidant potential

tumerone (8.46%), curlone (8.60%), 3-buten-2-one, 4-(4-hydroxy-3-methoxyphenyl), dehydrozingerone (35.82%) and ezlopitant, dehydro- (3.76%). The most abundant compound

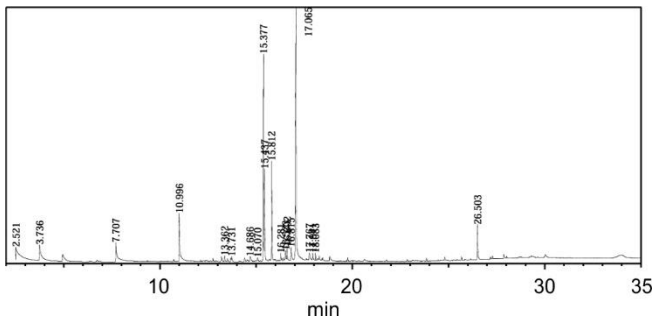

Fig. 4. GC-MS chromatogram of the ethanolic extract of *Curcuma longa*

was dehydrozingerone (35.82%), followed by aR-turmerone (18.29%), both of which are known bioactive constituents of turmeric with antioxidant and anti-inflammatory potential. Other compounds such as phenol, 2-methoxy- (guaiacol), 2-methoxy-4-vinylphenol, bisabolone and methyl palmitate were also detected in smaller proportions, contributing to the overall pharmacological activity of the extract. Thus, GC-MS profiling revealed that *C. longa* ethanolic extract is rich in turmerones and phenolic derivatives, which are responsible for its antioxidant activities.

GC analysis of hemp seed oil extract: On the basis of comparison of the mass spectra with the NIST library, a total of 10 compounds were identified in the GC-MS chromatogram of the hemp seed oil extract (Fig. 5; Table-9). The major compounds identified were linoleic acid (56.11%), linolenic acid (17.02%), oleic acid (13.64%) and palmitic acid (8.03%), which together constituted more than 90% of the total composition. Minor components such as stearic acid (2.98%), γ-linolenic acid (0.79%), arachidic acid (0.58%), *cis*-11-eicosenoic acid (0.35%), heneicosanoic acid (0.22%) and *cis*-8,11,14-eicosatrienoic acid (0.27%) were also detected in trace amounts. The predominance of polyunsaturated fatty acids (PUFAs), especially linoleic and linolenic acids, high-

TABLE-9
LIST OF COMPOUNDS IDENTIFIED IN HEMP SEED OIL EXTRACT BY GC-MS ANALYSIS

Peak	RT (min)	Area (%)	Compound name	m.f.
1	30.510	8.033	Palmitic acid	$C_{16}H_{32}O_2$
2	38.806	2.982	Stearic acid	$C_{18}H_{36}O_2$
3	40.424	13.644	Oleic acid	$C_{18}H_{34}O_2$
4	43.060	56.108	Linoleic acid	$C_{18}H_{32}O_2$
5	44.709	0.580	Arachidic acid	$C_{20}H_{40}O_2$
6	44.989	0.794	γ-Linolenic acid	$C_{18}H_{30}O_2$
7	45.773	17.019	Linolenic acid (α-linolenic acid)	$C_{18}H_{30}O_2$
8	46.183	0.349	*cis*-11-Eicosenoic acid	$C_{20}H_{38}O_2$
9	47.433	0.222	Heneicosanoic acid	$C_{21}H_{42}O_2$
10	49.932	0.268	*cis*-8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$

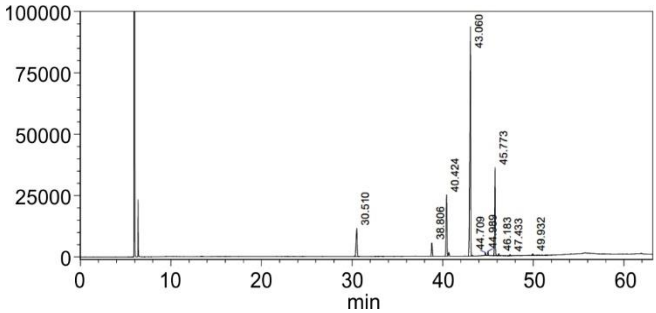

Fig. 5. GC-MS chromatogram of the hemp seed oil extract

lights the nutritional and therapeutic value of hemp seed oil, known for its antioxidant, anti-inflammatory and cardioprotective properties. The presence of both ω-6 (linoleic acid) and ω-3 (linolenic acid) fatty acids in an optimal ratio further underscores the oil's potential in promoting human health and preventing oxidative stress-related disorders.

The DPPH radical-scavenging assay of the combined extract exhibited concentration-dependent increases in % inhibition, from 65.7% at 40 μg/mL to 85.2% at 100 μg/mL, with corresponding IC_{50} values declining from 60.9 to 45.6 μg/mL. The trend of decreasing IC_{50} with increasing concentration highlights enhanced radical-quenching efficacy. This potent activity likely arises from synergy between *C. longa* phenolics (curcumin and derivatives) and hemp seed oil lipids. Curcumin and phenolic constituents directly scavenge DPPH and resonance stabilization. Meanwhile, minor phenolics, tocopherols, or antioxidants present in hemp oil may regenerate oxidized curcuminoid radicals, effectively improving turnover and sustaining antioxidant capacity. Indeed, studies have shown that incorporating curcumin into lipid-based carriers or emulsions enhances its stability and radical scavenging [41]. Furthermore, although linoleic and α-linolenic acids are not strong direct DPPH scavengers, they contribute to attenuation of lipid peroxidation and stabilization of radical chain reactions in lipid environments. In a combined system, the hydrophobic phase can facilitate better orientation and interaction of antioxidants with radical species–effectively bridging lipophilic and hydrophilic domains.

Conclusion

This study provides a comprehensive comparative evaluation of the antioxidant potential of *Curcuma longa* L. and *Cannabis sativa* L. extracts, underscoring their chemical diversity and therapeutic promise. Phytochemical and spectroscopic characterization revealed a rich presence of phenolics, curcuminoids, flavonoids, terpenoids and cannabinoids, each contributing through distinct radical scavenging and redox-modulating mechanisms. The DPPH assay confirmed that *C. longa* ethanolic and Soxhlet extracts exhibited strong antioxidant capacity, while *C. sativa* essential oil demonstrated moderate activity and hemp seed oil exerted comparatively weaker effects. GC-MS profiling further substantiated these results, identifying major antioxidant-related compounds such as dehydrozingerone, turmerones and curlone in *C. longa*, along with a predominance of polyunsaturated fatty acids (linoleic, α-linolenic and oleic acids) in hemp seed oil, highlighting their complementary roles in radical stabilization and lipid protection. Notably, the combined herbal formulation of *C. longa* and hemp seed oil showed marked improvement in scavenging efficiency, with reduced IC_{50} values, indicating significant synergistic interactions between phenolic-rich curcuminoids and polyunsaturated fatty acids. Significantly, the formulation combining *C. longa* ethanolic extract with *C. sativa* essential oil exhibited synergistic enhancement of antioxidant activity, reflecting the complementary chemistry of phenolic antioxidants and lipid-soluble terpenoids. Together, these findings validate traditional use of these botanicals and provide mechanistic insight into their chemical synergy, supporting their application in the design of polyherbal formulations and nutraceuticals for oxidative stress related disorders.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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