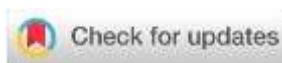


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Research Article

Phytochemistry and Antiperoxidative Potential of *Cannabis sativa* L. Leaves Methanol Extracts: An *In Vitro* Study

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Abstract

Aim: Present study aimed to demonstrate the preliminary phytochemical composition, antioxidant and in vitro protective potential of the methanolic extract of *Cannabis sativa* L.

Study Design: A methanolic extract of *Cannabis sativa* L. leaves was prepared and assessed at the *in vitro* level for its preliminary phytochemical screening and antiperoxidative potentials by using the DPPH radical reduction assay, lipid peroxidation inhibitory activity, protein carbonyl inhibitory potential in glycated bovine serum albumin, and enzyme antioxidant activity in a goat liver homogenate system exposed to hydrogen peroxide as oxidant molecule.

Results: The methanolic extract of *Cannabis sativa* L. leaf contained a diverse range of phytoconstituents, including alkaloids, flavonoids, tannins, terpenoids, and saponins. Upon quantitative analysis, the methanolic extract of *C. sativa* L. reflected 50 ± 0.013 μ g quercetin equivalent / mg of extract according to the quercetin standard graph, while 28.03 ± 0.024 % alkaloid content was retrieved after quantification.

Conclusion: We suggest that, upon tested on all *in vitro* antioxidant parameters; the leaf methanolic extract of *Cannabis sativa* L. demonstrated potent antioxidant and protective activity.

Keywords: *Cannabis sativa* L., Antioxidant, *in vitro*, DPPH, LPO, Protein carbonyl, peroxidative potential

INTRODUCTION

The Indian Subcontinent is known to possess a diversity of those plant species that have great medicinal importance and therapeutic history due to their rich phytochemical composition in the form of phenolic compounds such as alkaloids, flavonoids, tannins, coumarins, etc ^{1,2}. Traditional healers in India have used different parts of these herbs to prepare various natural preparations and their combinations since time immemorial, following an ancient medical ideology ^{3,4}. Different preparations made from the components and organs of these entities such as leaves, flower, stem, seeds, fruit, etc. are utilized as curative agents for many ailments mentioned in ancient Ayurveda literatures ⁵. Some of the important representative herbs with their consumable parts are Ashwagandha *Withania somnifera* (Root), Atees *Aconitum heterophyllum* (Rhizome), Bael *Aegle marmelos* (Fruit/Bark/Leaves), Tulsi *Ocimum tenuiflorum* (Whole Plant), Hemp *Cannabis sativa* (leaves), Musali *Chlorophytum borivilianum* (Rhizome), Kokum *Garcinia indica* (Fruit), Amla *Emblica officinalis* (Fruit), etc ⁶. Due to the potent antioxidant capabilities above mentioned naturales tends to cope as anti-diabetic, anti-obesity, anti-arthritic, anti-inflammatory, antibacterial, hepatoprotective, cardio protective, antidepressant and anxiolytic effects and so many other properties ⁷.

Since the alterations in metabolism either due to infectious or non-infectious conditions ultimately up sets the generation of free radicals or reactive oxygen and reactive nitrogen species, the later will causes the "Oxidative stress" ^{8, 9}. The current scenario of fast and unhealthy diets makes the system more susceptible and prone to oxidative stress, which will then set off various metabolic and physiological errors ^{10, 11}. According to the trend analysis of the last few years, a specific population has displayed a tendency towards the consumption of natural and herbal substances in their diet plan. It shows that natural substances are capable of reducing stress and related conditions ^{12,13}.

Cannabis sativa L. is one of the most valuable plant species, having both religious and medicinal value. Because of its connection with Lord Shiva, this herb has been regarded as a miracle plant since antiquity ¹⁴. The Cannabaceae (Marijuana Family) herb has a stem height of 6-12 feet with palmately split leaves that are 3-7 segments long and 3-6 cm long. It is a dioecious plant with fibrous and scrubous bark ¹⁵. Cannabinoids, a chemical found in hemp (commonly known as marijuana), are utilised to enhance the immune system and have an impact on both the peripheral and central nervous systems by acting as cannabinoid receptor CB2 ^{16,17}. It is classified as a multifunctional crop since several of its parts are used as medicines. It not only has the ability to cure a variety of diseases such as chronic pain, multiple sclerosis,

depression, arthritis, neuropathy, diabetes, and Parkinson's disease, but it also has an incredible antioxidant function. It not only treats the least fatal diseases, but some studies have indicated that it can help cure severe or lethal diseases such as sickle cell anaemia, ulcerative colitis, and others. Cannabis sativa aids in the reduction of oxidative stress (the production of reactive oxygen species such as superoxide) ¹⁸.

Looking after the therapeutic importance of *Cannabis sativa* L. covered in above mentioned literature, our present study was focused on the preliminary Phytochemical screening and antiperoxidative potentials of *Cannabis sativa* L. Leaves methanol extract under *in vitro* set ups.

MATERIALS AND METHODS

All the chemicals used in this study were of analytical grade and purchased from **Himedia** and **Merk Chemicals** India Ltd., while all the spectrophotometric analysis was carried out in a double-beam UV-Vis spectrophotometer by Lyzer Ltd. in India.

Collection of Plant material and preparation of extract

Dried leaves of *Cannabis sativa* L. were purchased from a licensed vendor of hemp in local market of Indore city India. Leaves were grinded with mortar and pestle and mixed with the solvent methanol. The methanol extract were obtained according to the method describes by **Audu et al., (2000)** ¹⁹ with slight modifications. Briefly a fraction 10g of the crushed leave of the plant was weighed out and soaked in 100ml of the methanol and kept for three days with occasional shaking to take out the extract. The extract was then filtered using Whatman no.1 filter paper. All filtrates are dried at 28°C for three days to obtain semi dried extracts.

Sample preparation for *in vitro* studies

A stock solution of concentration of 1000µg/ml of leaves extract was prepared in methanol while standard Ascorbic acid was prepared in 0.1 M phosphate buffer. Then both the solutions were kept in dark narrow mouth bottles and stored at 4°C.

Preliminary Phytochemical Screening:

Preliminary screening of Alkaloids, Saponins and terpenoids was done according to the standardized method described by **Harborne, (1998)** ²⁰, coumarins were detected according to the protocol by **Trease and Evans, (1989)** ²¹ while flavanoids and tannins were screened according to the standard procedure elucidated by **Sofawora, (1993)** ²².

Quantification of Phytoconstituents

Quantification of Total Flavonoid Content

The total flavonoid content (TFC) was calculated using an established method with minor modifications. 1 mL of sample (1 mg/mL) was combined with 4 mL of distilled water and 0.3 mL of 5% NaNO₂. After 5 minutes, 0.3 mL of 10% AlCl₃ was added and incubated for 5 minutes. The solution was then thoroughly mixed with 2 mL of 1M NaOH. After 30 minutes, the absorbance was measured at 510 nm against the blank solution. The flavonoid compound's quantification was done in triplicate. TFC was calculated as g of quercetin equivalents (QE/mg) of dried extracts using a quercetin calibration curve **Ribarova et al., (2005)** ²³.

Quantification of Total Alkaloid content

The total alkaloid content was measured using standardized method ²⁴. In a 250 ml beaker, 200 ml of 10% acetic acid in ethanol was applied to CS Methanolic extract (2.50 g) and left to stand for 4 hours. The extract was concentrated to one-quarter of its original volume in a water bath, then 15 drops of concentrated ammonium hydroxide were added dropwise to

the extract until the precipitation was complete immediately after filtering. After 3 hours of sedimentation, the supernatant was discarded, and the precipitates were washed with 20 cm³ of 0.1 M ammonium hydroxide and filtered through Whatman no.1 filter paper. The residue was dried in an oven and weighed with an automated weighing scale. % yield of total alkaloid was calculated using following formula

$$\% \text{ Alkaloid} = \frac{\text{Wt. of Alkaloid residue}}{\text{Wt. of Sample}} \times 100$$

Determination of Antioxidant activity

DPPH radical scavenging activity

DPPH is a nitrogen-free radical that becomes unstable in solution and has a violet color. Any antioxidant-active substance provides a proton to the unstable free radical DPPH, quenching the colour from violet to a less dense colour. To prevent DPPH radical autolysis, 0.004% DPPH radical solution was thoroughly mixed with 1 ml of plant extract solution ranging from 100 to 500 g/ml and incubated at room temperature in the dark for 30 minutes. After incubation, the radical concentration is measured at 517 nm as the antioxidant's percent inhibitory activity versus a control containing methanol and DPPH solution without plant extract. For baseline correction, methanol was used as a blank ²⁵.

$$\% \text{ Inhibition} = \frac{\text{O.D. Control} - \text{O.D. Sample}}{\text{O.D. Control}} \times 100$$

Inhibition of lipid peroxidation in egg yolk homogenate

Lipid peroxidation inhibitory potential of leaves extract was determined using egg yolk as a lipid-rich medium according to **Ruberto et al., (2000)**. A 10% V/V egg homogenate was produced in phosphate buffer saline (pH 7.4), and 0.1 ml of different concentrations of leaves extract were combined individually before volume was increased to 1 ml by adding phosphate buffer (solvent). To start the Fenton reaction, 0.1ml of FeCl₃ was added to the mixture and incubated for 30 minutes to stimulate lipid per oxidation. The mixture was then incubated for 10 minutes after 1.5 ml of TCA was added. After 10 minutes, 1.5 ml TBA was added to the mixture and it was placed in a hot water bath for 30 minutes. The pink tint signals the development of the MDA-TBA complex ²⁶. The absorbance of the organic top layer containing the complex was measured at 532 nm.

$$\% \text{ Inhibition} = \frac{\text{O.D. Control} - \text{O.D. Sample}}{\text{O.D. Control}} \times 100$$

Inhibition of protein carbonyl in BSA model system

Inhibition of protein carbonyl generation in glycated BSA system was evaluated according to method standardized by **Martinez et al., (2001)** ²⁷. In brief BSA was dissolved in phosphate buffered saline (pH 7.4) to make a 10mg/ml stock solution. This solution was subsequently diluted with fructose stock solutions prepared in PBS to make triplicate. The plant extract was then added to the mixture in concentrations ranging from 10 g/ml to 50 g/ml, and the volume was increased to 1 ml by adding distilled water. The reaction mixtures were then incubated at 37°C for 4 days. DNPH (2,4-dinitrophenyl hydrazine) was combined into 0.5 ml of glycated sample. Incubate the reaction mixture at room temperature for 1 hour. After that, add 0.5 mL of TCA (Tri chloro acetic acid). The centrifuge was set to 10,000 rpm for 30 minutes at 4°C. Pellet rinsed three times with an ethanol:ethyl acetate solution and 1ml urea was used to dissolve the pellet. The optical density was measured at 365 nm.

$$\% \text{ Inhibition} = \frac{\text{O.D. Control} - \text{O.D. Sample}}{\text{O.D. Control}} \times 100$$

Determination of the activity of enzymatic antioxidants in Goat liver slice system

Goat liver Homogenate preparation

Fresh goat liver was purchased from a local slaughterhouse. The tissue was rapidly immersed in cold PBS and kept at 4°C. With a sterile scalpel, the tissue was sliced into 1 mm thin slices. 250 mg of tissue were dissolved in 1.0 mL of cold PBS. 5 µl of 200 µM H2O2 and 5 µl methanolic extract were added and incubated for one hour at 37°C with gentle shaking. Following the incubation period, the tissues were homogenised with a Teflon homogenizer in the same amount of PBS and centrifuged to remove debris. The supernatant was then utilised to calculate various parameters to determine the antioxidant potential.

The experimental groups set up for the study were as follows.

A. Untreated (negative) control.

B. H2O2 treated (positive) control.

C. Methanolic extract of Cannabis Sativa leaves+H2O2

The antioxidant activity of Cannabis sativa leaves was determined by evaluating the activities of enzymic antioxidants.

Assay of catalase (CAT)

Catalase activity was measured using Luck's, (1974) ²⁸ method. 3 ml of H2O2 in phosphate buffer (0.067M, pH 7.0) was placed in a quartz cuvette, and the baseline was adjusted at 240 nm before adding 20 l of homogenate and thoroughly mixing. At 240 nm, the time interval for a 0.05 unit drop in absorbance was recorded. The enzyme supply and phosphate buffer were used as controls. The amount of enzyme necessary to reduce the absorbance at 240 nm by 0.05 units was determined as one enzyme unit.

Assay of peroxidase (POD)

The activity of peroxidase in the samples was determined using the method developed by Reddy *et al.*, (1995) ²⁹. Pipette out 3.0 mL of pyrogallol solution and 0.1 mL of enzyme extract into a cuvette. The spectrophotometer was set to zero at 430 nm, and then 0.5 mL of 1% H2O2 was added and stirred. The absorbance change was recorded every 30 seconds for up to 3 minutes. The change in absorbance per minute at 430 nm is defined as one unit of peroxidase activity.

Statistical analysis

All the tests were carried out in triplicates and calculations were done as mean values. Data was expressed as Mean ± S.D.

RESULTS

Methanolic extract of *C. sativa* leaves was evaluated at the level of Phytochemistry; qualitative as well as quantitative. Antioxidant assessment in vitro and enzyme antioxidant parameters also at in vitro level according to standardized methods of phytochemical and antioxidant analysis mentioned in literature. Results revealed the presence of important phytoconstituents as secondary metabolites which were later favored the tremendous antioxidant and protective potential of *C. sativa* leaf methanol extract. It was indicated that adequate presence of secondary metabolites (Phenolic compounds) directly correlated with the antioxidant activity of the test sample.

Phytochemical Screening

The presence of alkaloids, coumarins, flavonoids, saponins, tannins, and terpenoids was detected in a methanol extract of Cannabis sativa leaves. Results were expressed as the sign of

(+) as an indicator of presence. Flavanoids and alkaloids were detected as strong impressions (+++ i.e. dense color complex or reaction was formed) in qualitative assessments, while others were found as moderate impressions (+/++ Mild color complex or reaction was formed) based on their detection criteria. (Table 1)

Table 1: Qualitative screening of Phytochemicals in methanolic extract of *C. sativa* leaves.

S. No.	Phytoconstituents screened	Result
1	Alkaloid	+++
2	Tannin	++
3	Saponin	++
4	Terpenoid	+
5	Flavanoid	+++
6	Coumarine	++

Note: Degree of detection showed as + Low, ++ Mild and +++ High content

Quantitative assessment of phytoconstituents

Total flavanoid content

A calibration plot was prepared for quercetin as standard flavanoids at the concentration range between 10, 25, 50, 75 and 100 µg/ml ($y = 0.000x + 0.003$, $R^2 = 0.962$) which was used to quantify the value of total flavanoid content in the test extract as µg Quercetin equivalent / mg of extract (Figure 2). Here Methanolic extract of *C. sativa* exhibited 50 ± 0.013 µg Quercetin equivalent / mg flavanoid content. (Table 2)

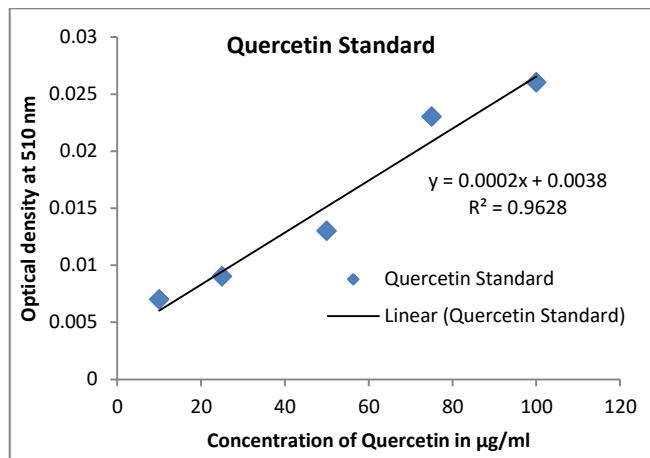


Figure 1: Linear regression graph of Quercetin at various concentrations viz 10 to 100 µg/ml on 510 nm as a standard for determination total Flavanoid content.

Table 2: Total flavonoid content in *C. sativa* leaf methanolic extract according to µg/ml Quercetin equivalent. Data expressed as Mean ± S.D.

S. No.	Name of Phytoconstituents	Quantity as µg Quercetin equivalent / mg of extract
1	Total Flavanoid	50 ± 0.013

*All results in triplicates.

Total Alkaloid content

A standard method of quantitative analysis of Total alkaloid content was applied for the methanolic extract of *C. sativa*

leaves where the residual content after filtration was measured at 28.03 ± 0.024 % total alkaloid content obtained from the test extract. (Table 3)

Table 3: Total Alkaloid content in *C. sativa* leaf methanolic extract at % yield. Data expressed as Mean \pm S.D.

S. No.	Name of Phytoconstituents	% of Total alkaloid Content
1	Total Alkaloid content	28.03 ± 0.024

*All results in triplicates.

DPPH radical scavenging assay

DPPH radical scavenging activity of test extract was evaluated in comparison of a standard ascorbic acid at various concentrations 10, 200, 300, 400 and 500 $\mu\text{g}/\text{ml}$. Both the ascorbic acid and *C. sativa* methanolic extracts showed concentration dependent increase in the % inhibition of DPPH radical, where test extract reflected highest activity 94.62 ± 0.746 at 400 $\mu\text{g}/\text{ml}$ and after that a decrease in antioxidant activity was recorded while ascorbic acid showed highest radical scavenging activity 87.69 ± 0.49 at 500 $\mu\text{g}/\text{ml}$. Result reflected that methanolic extract of *C. sativa* leaves was potent enough to stabilize the nitrogen free radical DPPH system but at much higher concentration it behaves as a prooxidant since radical reduction % was dropped at 500 $\mu\text{g}/\text{ml}$. (Figure 2)

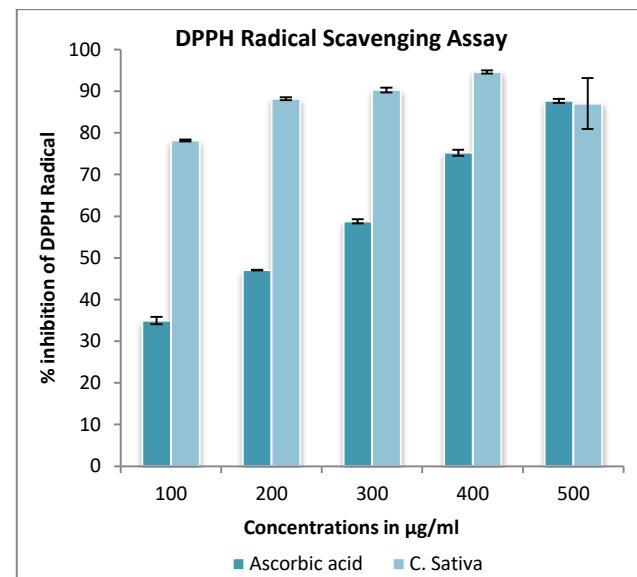


Figure 2: Inhibition of DPPH radical by Ascorbic acid and methanolic extract of *C. sativa* leaf at various concentrations viz100, 200, 300, 400 and 500 $\mu\text{g}/\text{ml}$. Data expressed as Mean \pm S.D. (n=3)

Inhibition of Lipid peroxidation in Egg yolk system

Egg yolk; a lipid rich medium; was served as a system to induce Lipid peroxidation through fenton reaction, and the inhibition of which was evaluated using methanolic extract of *C. sativa* and standard Ascorbic acid at varying concentrations viz100, 200, 300, 400 and 500 $\mu\text{g}/\text{ml}$. Here again test extract and ascorbic acid reflected dose dependent increase in the inhibition of lipid peroxidation in egg yolk system where at 250 $\mu\text{g}/\text{ml}$ a highest inhibitory potential of 60.99 ± 0.16 was obtained for *Cannabis sativa* extract in comparison of 67.84 ± 0.45 for Ascorbic acid standard at same concentration. (Figure 3)

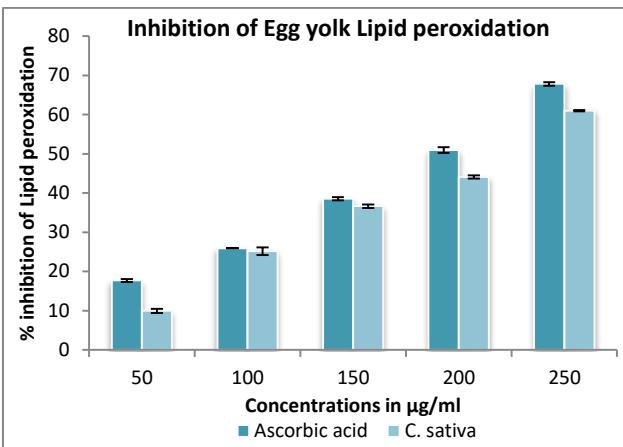


Figure 3: Inhibition of Lipid peroxidation in Egg yolk System by Ascorbic acid and methanolic extract of *C. sativa* leaf at various concentrations viz50, 100, 150, 200 and 250 $\mu\text{g}/\text{ml}$. Data expressed as Mean \pm S.D. (n=3)

Inhibition of protein carbonyl in Glycated BSA

The criteria of preventing generation of protein dinitrophenylhydrazone moiety mediated by the inhibition of protein carbonylation in BSA model glycated with fructose was assessed for test extract of *Cannabis sativa* leaves with a dose dependent increase in inhibitory potential from concentration ranging 10 to 100 $\mu\text{g}/\text{ml}$. Initiated as lowest % inhibition of 58.86 ± 0.15 at 10 $\mu\text{g}/\text{ml}$ to reaching highest % inhibition of 93.4 ± 0.42 at 100 $\mu\text{g}/\text{ml}$. Results clearly indicates the molecule protective and ameliorative potential of the test extract as it inhibited to react dinitrophenyl hydrazine with protein and subsequently prevented the generation of oxidant dinitrophenylhydrazone. (Figure 4)

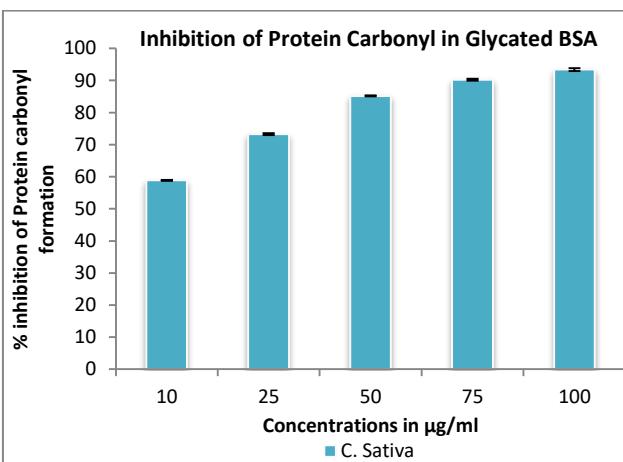


Figure 4: Inhibition of Protein carbonyl BSA System glycated with Fructose by Ascorbic acid and methanolic extract of *C. sativa* leaf at various concentrations viz10, 25, 50, 75 and 100 $\mu\text{g}/\text{ml}$. Data expressed as Mean \pm S.D.

Analysis of Enzyme antioxidant activities

The current investigation found that the activities of enzymic antioxidants were significantly lower in the H₂O₂ exposed group compared to the untreated control group. When compared to the untreated control group, treatment with a methanolic extract of *Cannabis sativa* leaf resulted in a significant improvement in antioxidant status. The antioxidant levels in the oxidant and plant extract treated group were higher than in the oxidant alone treated group, indicating that the leaf extract has a protective role in oxidatively challenged goat liver slices. Table 1 shows the actions of enzymic antioxidants.

Table 3: Estimation of Enzyme antioxidant activity in Goat liver slice system treated with H2O2 and methanolic extract of C. sativa leaf. Data expressed as Mean \pm S.D. in enzyme activity unit/gram tissue.

S. No.	Enzyme Activity assay U/g Tissue	Normal Control (Goat liver slice + D/W)	Positive Control (Goat liver Slice + H2O2)	Treated group (Goat liver slice + H2O2 + C. sativa extract)
1	Catalase	367.00 \pm 11.07	145.83 \pm 37.53	307.92 \pm 21.77
2	Peroxidase	31.12 \pm 0.014	25.78 \pm 0.0032	29.40 \pm 0.034

DISCUSSION

Since ancient times Folklore therapists in South India and entire asia used medicinal plants to heal various diseases; they imply therapeutic characteristics of medicinal plants for curing skin problems, poison bites, stomach ache, and psychological disorders ³⁰. Yet, in some countries, such as Bangladesh, the medicinal plant is also used to cure UTIs (Urinary Tract Infections) and STDs (Sexually Transmitted Diseases) ³¹. Simillarly, ethopharmacists and ethnovets in the the Pauri Garhwal District in Uttrakhand uses herbs to cure both human and veterinary diseases ³². Likewise, several cultures recognise *Cannabis sativa* for its medical properties. Its intricacy leads to the historical use of numerous plant components in ethnomedicine and pharmacology ^{33, 34}. Bioactive phytochemicals are active therapeutic plant components. These bioactive molecules are thought to improve plants' ability to survive or adapt to their surroundings, and they are utilised in humans as medications, flavourings, and recreational substances ³⁵.

Cannabis sativa L. has been used for medical purposes since ancient times due to its abundant supply of phytochemicals, prompting scientists to explore its pharmacological potential ³⁶. Investigation in present study revealed the abundance of important phytoconstituents especially total flavanoids and alkaloids which are considered as the chief components to determine antioxidant and protective roles. **Audu et al., 2014** ³⁷, reported presence of alkaloids, flavonoids, cardiac glycosides, resins, terpins and steroids in the crude extract of *C. sativa* leaves which were later observed also by **Choudhary et al., 2014** ³⁸ and **Sharma et al., 2022** ³⁹ in extracts of leaves, root and stem of *Cannabis sativa*. Upon quantification for phytoconstituents, we obtained 50 ± 0.013 total flavanoid and 28.03 ± 0.024 % total alkaloid content in the test methanolic extract similarly **Ahmed et al., 2019** ⁴⁰ reported abundance of alkaloids, tannins, terpenoids and phenolics and a total flavanoid content of 59.03 ± 1.312 to $\mu\text{g/ml}$ Quercetin equivalent in leaf methanolic extract of *Cannabis sativa*.

Free radicals have been linked to a wide range of clinical symptoms. Antioxidants attack free radicals and protect us from a variety of ailments. They work by either scavenging reactive oxygen species or preserving antioxidant defence systems ⁴¹. The ability of natural products to donate electrons can be tested by quenching a purple-colored solution with 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH). The approach works by reducing DPPH by adding a radical species or antioxidant that decolorizes the DPPH solution. The degree of colour change is proportional to the antioxidant content and strength ⁴². The present study findings on the free radical stabilising activity of *Cannabis sativa* leaf methanolic extract upon the DPPH system revealed its strong antioxidant potential, as evident by extensive studies for free radical reduction capability in different solvent extracts of various components of *Cannabis sativa* ^{43,44,45}.

It has been proposed that lipid peroxidation occurs via a free radical chain reaction, which has been linked to cell damage in biomembranes. It has been demonstrated that the damage

causes several ailments such as cancer, cardiovascular disease, and diabetes. The addition of FeSO₄ induces a considerable increase in lipid peroxidation in egg yolk homogenates. Because we employed egg yolk as a substrate, it is possible that *Cannabis sativa* is active against nonenzymatic oxidation. **Hussain et al., 2021** ⁴⁴ reported *in vitro* lipid peroxidation inhibitory potential of *Cannabis sativa* in goat liver and brain homogenates.

Irreversible kind of protein modification produces Protein carbonyls that have been shown to be very durable (degradation and clearance in hours or days), in comparison to lipid peroxidation products that are eliminated in minutes. Furthermore, protein carbonyls are produced early during oxidative stress conditions and are not caused by a single oxidant, so they can be considered a measure of overall protein oxidation. We have showed inhibitory potential of *Cannabis sativa* leaves methanolic extract against *in vitro* protein carbonyl generation in glycated BSA model whereas some other studies reflected inhibition of advance glycation end products and advance oxidative protein products to demonstration prevention of protein carbonyls by other plant species. ^{46,47}.

Several conditions, including drought, cold, heat, herbicides, and heavy metals, produce reactive oxygen species (ROS), which increase the amount and concentration of ROS in plant cells. Catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase are enzymatic antioxidant systems that work together to reduce oxidative damage caused by reactive oxygen species ⁴⁸.

Catalase is a tetrahedral protein with four heme groups that catalyses hydrogen peroxide dismutation in water and oxygen. Diabetes, vitiligo, cardiovascular disease, Wilson disease, hypertension, anaemia, several dermatological conditions, Alzheimer's disease, bipolar disorder, and schizophrenia are all linked to catalase shortage or dysfunction ⁴⁹. Peroxidase enzymes catalyse oxidation-reduction reactions via the free radical mechanism, converting a variety of chemicals into oxidised or polymerized products ⁵⁰. The current study's results on catalase and peroxidase activity in H2O₂ exposed goat liver homogenate demonstrated the corrective ability of *C. sativa* leaf extract when compared to the normal control and positive control which reflects its oxidant inhibitory as well as ameliorative potential for enzymatic antioxidant systems. **Kubiliene et al., 2021** ⁵¹ reported significant increase in hepatic catalase activity in male BALB/c received *Cannabis sativa* extract 20 min before the exposure of various oxidants. Similarly it has been observed that acatalasemia, an uncommon genetic illness, is inherited with a catalase activity abnormality (also known as Takahara disease) ^{52, 53}. Catalase is essential for regulating the cellular amount of hydrogen peroxide.

CONCLUSION

Cannabis sativa L. a plant with miscellaneous therapeutic characteristics and bioactive compounds has been consumed

since decades to treat various ailments either associated with metabolism, infection, psychosis etc. Our present study showed a very preliminary composition of phytochemicals in the methanolic extract of *Cannabis sativa L.* leaves. The study also included an assessment of its free radical quenching potential, biomolecule protective ability, and the protection or amelioration of antioxidant enzyme biomarkers from oxidative damage in the goat liver system. Across all test parameters, *C. sativa* extract demonstrated its ability to stabilise free radicals as well as protect tissue enzyme biomarkers from the effects of damaging oxidative agents in *in vitro* systems. It can be said that this extract decreases free radicals and ROS levels while raising the generation of molecules capable of protecting against oxidative stress by increasing the activity of antioxidant enzymes. Overall, *C. sativa* looks to be a feasible source of antioxidants that can be utilised therapeutically to address the issue of metabolic diseases caused by oxidative stress. These findings could be useful in the creation of new medications. Furthermore, analysis of bioactive components, viz., various cannabinoids, cannabidiols, and cannabidiolic acid, could be purified and investigated for their antimicrobial, *in vitro*, *in vivo*, *in silico*, preclinical, and clinical-level therapeutic roles.

AUTHOR CONTRIBUTION

Both authors of this article have equally contributed towards the completion of this study. Author AS accomplished the overall experimental analysis and the methodology writing under the supervision of author NP, who prepared and polished the complete article.

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