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Research paper

Antioxidant Analysis in Seeds of Four Different Accessions of Cannabis sativa L. from Jammu

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Abstract

Cannabis belonging to the *Cannabaceae* family is a medicinally important plant. In ancient times this plant was used for food, fibre and medicine. In recent times, the focus has gone back on the medicinal importance of this wonder plant. The seeds of the *Cannabis* contain proteins, vitamins, fatty acids and oil that provide antioxidant value and are nutritionally important. The current study was envisaged in order to evaluate the antioxidant potential of *Cannabis* seeds of different areas of Jammu. Results suggested that *Cannabis* seeds have phenol content ranging from 77.64 µg GAE 100 mg^{-1} DW to 103.62 µg GAE 100 mg^{-1} DW and flavonoid content ranging from 34.52 µg QR 100 mg^{-1} DW to 47.12 µg QR 100 mg^{-1} DW across the different accessions. Further, the antioxidant assays including FRAP, DPPH and chelating power assays suggested significant antioxidant activities in the seeds of accessions collected from Jammu. This is the first report of antioxidant evaluation from *Cannabis* seeds from Jammu area.

Keyword: *Cannabis sativa*, Antioxidants, Phenols, Flavonoids, DPPH free radical assay, FRAP assay, Chelating power assay.

Introduction

Seeds (legumes, cereals, oilseeds, etc) which contain macro and micronutrients, fibers, antioxidant compounds play an important role in the human diet with health and disease prevention benefits. Due to the nutritional and health beneficial properties of seeds, there is an increase in the use of non-conventional foods in past few years among people as they are changing their dietary habits to improve and maintain health status. The importance of these non-conventional foods which contain nutraceuticals and other natural active compounds has promoted health and reduced the risk to disease [1,2].

Cannabis belonging to the *Cannabaceae* family which is originated in central Asia is a wind-pollinated annual plant and is known for its long, thin flowers and spiky leaves [3,4,5]. It has been an important source of food, fibre, medicine, and psychoactive drug since ancient times. The two-main species include *Cannabis sativa* L. and *Cannabis Indica* L. Differentiating between the two species has been a long debate between the botanists

and differentiating between the two was a quite difficult task for the botanists [6]. *Cannabis* is one of the oldest plants used for the welfare of humans. In ancient times, china used this plant to produce cloth and fibre [7]. Since then, the uses of *Cannabis* reached the medical approach for humans by treating human disease in china. Ancient Egyptians use *Cannabis* for numerous problems like infections, analgesic, anti-inflammatory. The anxiolytic effects of *Cannabis* have been noted down in Vedic texts from India around 1500 BCE [8]. Since then, the medical benefits are noted throughout Asia and North Africa for many ailments. In the late 19th century, its use for the treatment for epilepsy in the children was reported using the THC isomer from *Cannabis* [9].

Research interest in *Cannabis* seeds has inclined due to the oil derived from it, which is used in the food and cosmetic products. It has been evaluated that the ripened seed of hemp and seed meal are excellent sources of dietary oil, fibre and protein [2,10]. The seed oil is known to be a rich source for unsaturated fatty acids [11,12]. Furthermore, the seeds of *Cannabis* contain protein, carbohydrates, insoluble fiber, vitamins, and minerals [10, 13]. As there is an increase in demand for new vegetable oils and demand of nutraceuticals for the welfare of the human being, there is a need to evaluate the non-conventional plant sources to achieve that. It is reported that the seeds are rich in all essential amino acids and fatty acids that are required for balanced dietary purposes and to improve human health [13, 14]. There are various reports available that show the principal component of *Cannabis* seeds lies in the content of essential and other fatty acids which makes it a good source of nutraceuticals [15,16].

Defending the body against free radical's attack, antioxidants play an important role by delaying or inhibiting the oxidation of lipids and other biomolecules thus, avoiding or repairing the damage to cells [17]. During energy metabolism and by environmental fall, inapt diets and contact to irradiation and stress, free radicals are generated (Packer and Colman, 1999) and there is solid evidence that these free radicals harm the lipids, cells, and proteins which can cause numerous irregularities in humans [18]. It has been reported that the seeds of *Cannabis* have a good quantity of flavonoids, tocopherols, flavanols and phenols which show better antioxidant activities against the free radicals [19].

This study was conducted in order to evaluate the antioxidant importance of *Cannabis* seeds of Jammu region. The study will help to scientifically validate the antioxidant and nutritional importance of the *Cannabis* seeds.

Materials and methods

All chemicals and reagents used in this study were purchased from Ms/-Himedia co, Ms/-Fisher Scientific co, Ms/-Sigma-Aldrich co, and M/s- SRL co.

Plant seed material

Cannabis seeds were collected from the different locations of the Jammu area as shown in table 1. The seeds were then bought to CSIR- Indian Institute of Integrative Medicine, Jammu and kept at 4°C in the dark area till further processing.

Table 1. Description of sites with DMS coordinates and sample codes from where seeds were collected in Jammu area. The seeds were stored at 4 °C in dark bag till analysis.

S. No.	Sample Code	Site	Altitude	Co-ordinates
1.	C1	Jammu	324	N 32^{0}46′32.11″ E 74^{0}48′23.0″
2.	C2	Parade, Jammu	270	N 32^{0}43′51″ E 74^{0}46′52″
3.	C3	Sanka Morh, Jammu	285	N 32^{0}44′30″ E 74^{0}48′09″
4.	C4	Parnalla, Billawar	712	N 32^{0}33′38″ E 75^{0}35′37.9″

Extract Preparation

The extract from *Cannabis* seeds was prepared using methanol according to the protocol suggested by Kaur et al. [20]. Briefly, Briefly, 100 mg seeds of each accession were crushed and dissolved in 1 ml methanol and centrifuged for 10 min at 10,000 rpm. The extraction was repeated three times and the supernatant was collected in a round bottom flask. Methanol was dried using the rotatory evaporatory system at 40 °C. The extract was weighed and dissolved in 1 ml of methanol for further studies. The methanolic extract was further diluted (1:10 v/v) before its use in assay procedures.

Total Phenol content

Diluted methanolic extract (100 µL) was mixed with 100 µL of 1 N Folin–Ciocalteu reagent followed by incubation for 5 min in dark to which 200 µL of 20 % Na_2CO_3 was added. Absorbance at 730 nm was measured in plate reader after 10 min incubation in dark and the concentration of phenolic compounds was calculated using the standard curve of gallic acid (0.5–5 µg; R^2 = 0.999). The results were expressed as gallic acid equivalent (µg GAE 100mg^{-1} DW).

Total flavonoid content

Diluted methanolic extract (100 µL) was mixed with 30 µL of a 5 % $NaNO_2$ solution and then incubated for 5 min, then after 300 µL of 10 % $AlCl_3.H_2O$ solution was added followed by addition of 200 µL of 1 M NaOH and 200 µL of distilled water after 6 min. Absorbance was read at 510 nm in a plate reader and total flavonoids were calculated using quercetin as standard (10–100 µg; R^2 = 0.999). The results were expressed as quercetin equivalent (µg QR 100mg^{-1} DW).

Antioxidant assays

DPPH free radical inhibition assay

Diluted methanolic extract (100 µL) was mixed with 50 µL of 0.5 mM DPPH radical solution prepared in methanol followed by addition of 100 µL of 0.1 M sodium acetate buffer (pH 5.5). Then incubated in the dark for 30 min followed by absorbance at 517 nm. Methanol was used as a negative

control and the radical scavenging activity (RSA) was calculated using the equation:

% DPPH radical scavenging activity = [(A_0 - A_s) / A0] × 100 Where A_0 is the absorbance of the control and A_s is the absorbance of the plant extract.

Reducing power assay

Undiluted methanolic extract (10 µL) was mixed with 250 µL of 0.2 M phosphate buffer (pH 6.6) and 250 µL of 1 % potassium ferricyanide (K_3Fe [CN]$_6$). The mixture was kept for the incubation at 50 °C for 20 min. To this, 10 % trichloroacetic acid was added and centrifuged at 1500 rpm for 10 min. The upper layer of the solution mixture (250 µL) was mixed with 240 µL of distilled water and 250 µL of 0.1 % ferric chloride solution. Absorbance was taken at 700 nm and ferric reducing/antioxidant potential was determined against the standard curve of $FeSO_4.7H_2O$ (0-0.5 µmoles; R^2 = 0.971).

Chelating power by Fe^{2+}

Diluted methanolic extract (100 µL) was further diluted with 100 µL of methanol and mixed with 20 µL of 1mM $Fecl_2$. The reaction was initiated by the addition of 40 µL of 5 mM Ferrozine solution followed by incubation in dark at room temperature for 10 min. Absorbance was then read at 562 nm. Methanol was used as a negative control and the chelating power was calculated using the equation:

% chelating power = [(A_0 - A_s) / A0] × 100 Where A_0 is the absorbance of the control and A_s is the absorbance of the plant extract.

Statistical Analysis

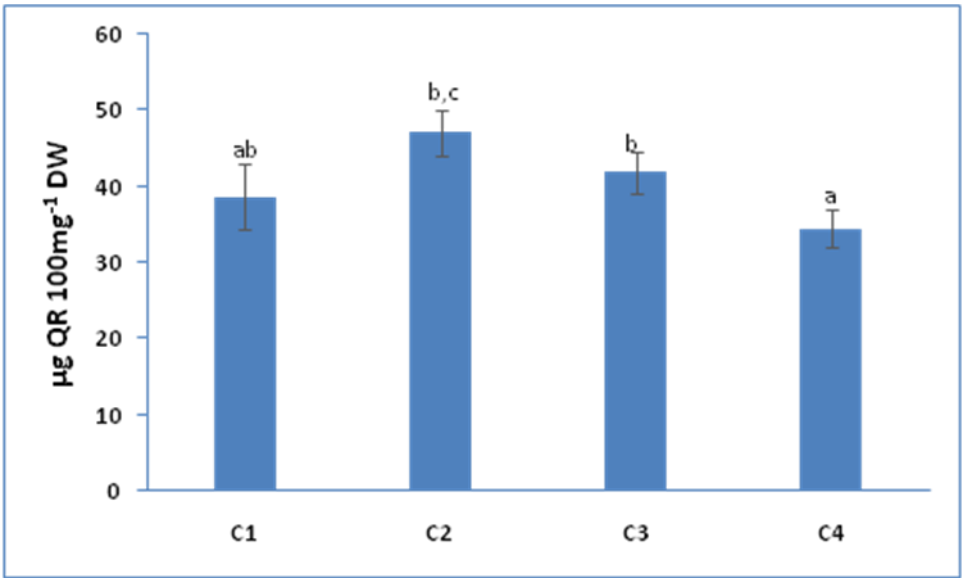
Two independent biological repeats and three technical repeats were performed and analyzed by One-way ANOVA followed by Tukey HSD *post hoc* test using IBM SPSS Statistics 25 Software, USA to identify the significant differences at α level of 0.05.

Results and Discussion

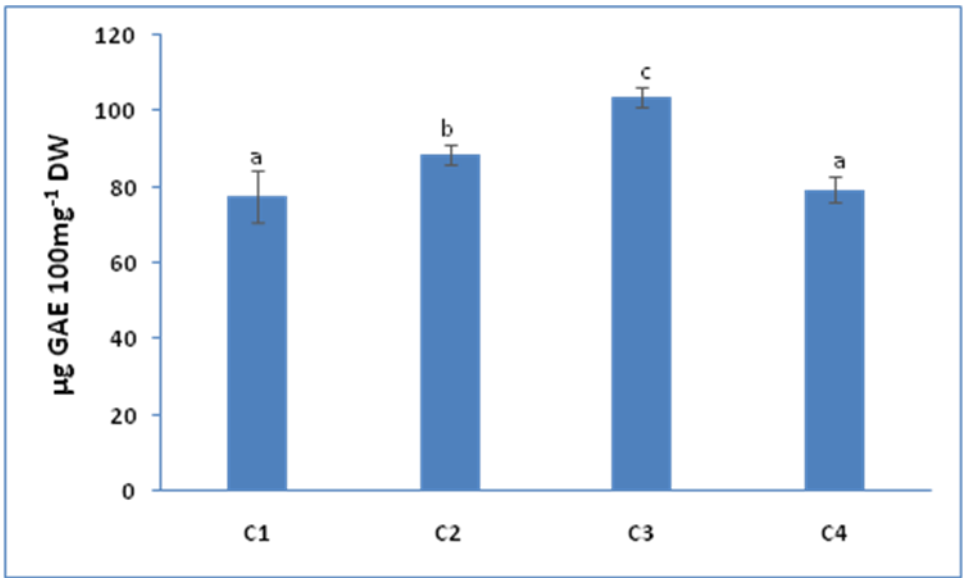
Total phenol and flavonoid content

Plant-derived secondary metabolites include phenolic compounds that have the capability of antioxidant activities [21] . In order to protect food products against oxidation, there are reports which show that phenolic compounds are being used as natural food additives [22]. These phenolic compounds also have numerous capabilities with cardioprotective and anti-inflammatory effects [21]. Folin–Ciocalteu reagent measures the ability of any mixture to reduce phosphomolybdic and phosphotungstic acids to a blue complex 23]. Total phenol content of all the accessions was done to find the redox properties of *Cannabis* seeds, as phenolic compounds present in plant material show redox properties. The total phenolic content ranged from 77.64 µg GAE 100 mg^{-1} DW to 103.62 µg GAE 100 mg^{-1} DW as shown in figure 1A. The highest phenol content was found in the C3 accession with 103.62 µg GAE 100 mg^{-1} DW.

Figure 1



A



B

Figure 1. Total phenol (A) and flavonoid (B) content in the seeds of *Cannabis* from different locations of Jammu area. The concentrations are expressed as µg GAE 100 mg^{-1} DW and µg QR 100 mg^{-1} DW, respectively. Different alphabet represents statistically significant values at ($p \leq 0.05$) as determined by one-way ANOVA with 'a' representing the minimum value.

There are reports which show that the total phenolic content of cold-pressed hemp seed oil extract is 44.0 mg 100 g^{-1} GAE [24]. In addition, Chen et al. [19] evaluated two

hemp accessions and reported the highest phenolic content of 1194 mg 100 g $^{-1}$ GAE (defatted kernels) and 10,920 mg 100 g $^{-1}$ GAE (defatted hull). Similarly, other reports submitted by Teh and Birch [25] found total phenolic acid content of 188.23 mg 100 g $^{-1}$ GAE in hemp seed oil. Another study that expressed total phenolic content of seeds extract based on both fresh weight and dry weight ranged from 2.21 mg GAE g $^{-1}$ and 2.33 mg GAE g $^{-1}$ respectively [7].

Another type of secondary metabolites that include flavonoids that have been found to possess numerous biological properties which include antioxidant, anti-allergenic and antiproliferative properties [26]. It has been studied that the flavonoids which show significant antioxidant activities in-vitro also exhibits the same trend when done in-vivo [27,28]. In the present study, total flavonoid content ranged from 34.52 µg QR 100 mg^{-1} DW to 47.12 µg QR 100 mg^{-1} DW. The highest flavonoid content was found to be in C2 accession with 47.12 µg QR 100 mg^{-1} DW followed by C3 accession with 41.89 µg QR 100 mg^{-1} DW (Figure 1B).

Antioxidant Assays

DPPH inhibition assay

In order to evaluate the antioxidant activity of phenols in the sample, DPPH is employed due to its constancy and suitability. DPPH• is a stable organic free radical which can lose chromophore when receiving a proton from any hydrogen donor. The DPPH % inhibition across the samples ranged from 44.46 % to 49.33 %. The highest % inhibition was found to be in the C4 accession with 49.33 % inhibition (Figure 2). Statistically significant differences in activity were measured by the DPPH• method. Reports submitted by Siger et al. [29] have shown over 50 % of DPPH activity in hemp seed oils. Another study by [7] also evaluated the DPPH inhibition using different solvent systems and it was found that the seeds have a good percentage of DPPH activity. Frassinetti et al. [2] evaluated seeds and sprouts of *Cannabis* to check the DPPH antioxidant activity and it ranged from 40 % to 52 % respectively.

Figure 2

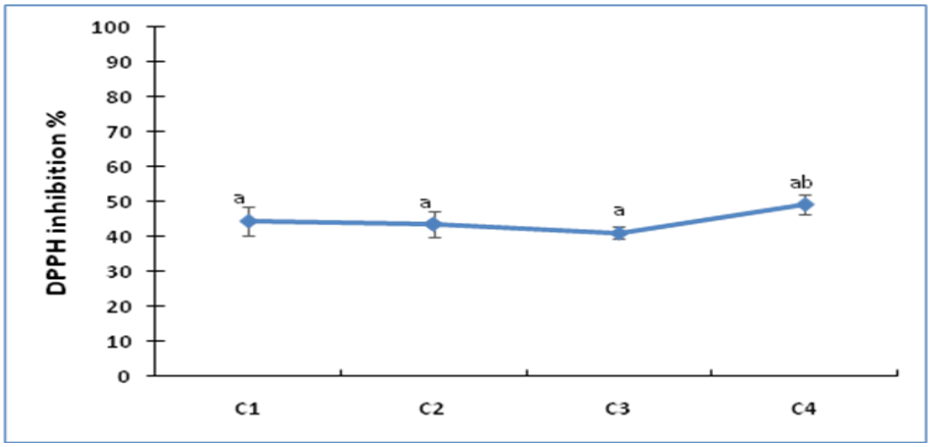


Figure 2. DPPH % inhibition activity of *Cannabis* seeds from Jammu area. Different alphabet represents statistically significant values at ($p \leq 0.05$) as determined by one-way ANOVA with 'a' representing the minimum value.

Reducing and chelation power

Other antioxidant activities were done to check the capability of phenols to reduce the ferric ions in the sample. Results suggested that all the samples show good reducing power (FRAP) which ranged from 15.89 to 26.24 µm FeSo4 100 mg^{-1} DW. The highest value was found to be in the C3 accession with value of 26.24 µm FeSo4 100 mg^{-1} DW (Figure 3). Chelating power assay was also conducted to check the capability of reducing the free ions in the sample and it was found that the chelating power percentage ranged from 12.68 % to 30.21 % across the samples. The highest chelating percentage was found to be in C1 accession which showed 30.21 % chelating capability (Figure 4). Though the seeds of C3 accession showed less chelating power as compared to other accessions, the probable reason might be due to compounds not reactive towards the assay. Smeriglio et al. [30] conducted experiments on the fraction of cold-pressed seed oil from Finola cultivars and the results suggested that the Finola cultivar showed higher antioxidant activities of FRAP and Chelating power. Their data suggested that the seeds of *Cannabis* may prevent oxidative damage from free radicals. *Quinoa* Seeds of different accessions from Ontario were evaluated for various antioxidant activities and it was reported that the seed was rich in reducing the ferric ions in the extracts. The values were of total phenol content were positively correlated with each other [32,33]. Another study was done by Dini et al. (2008) on red onion seeds also showed a strong correlation of phenolic content with the antioxidant activities.

Figure 3

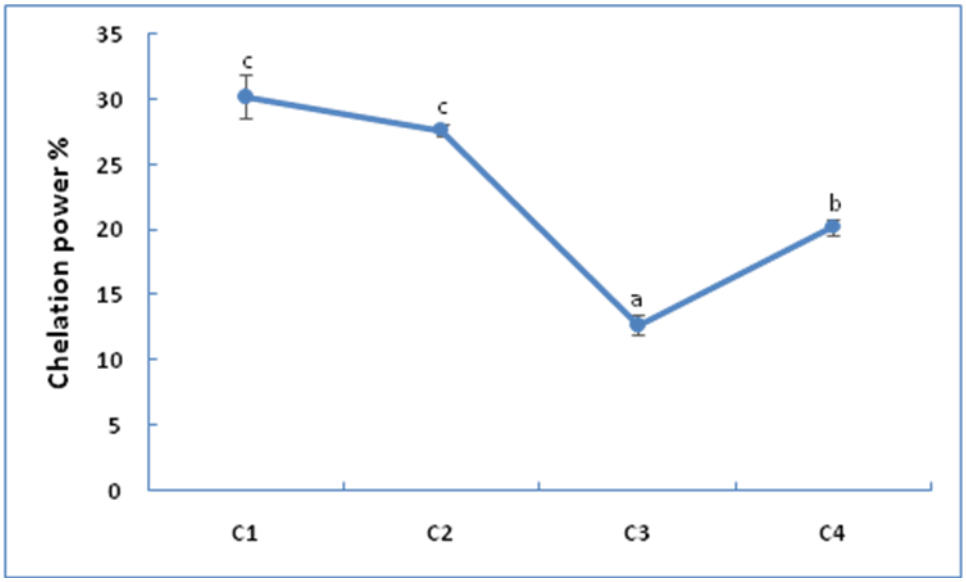


Figure 3. Reducing power assay of *Cannabis* seeds from Jammu area. Different alphabet represents statistically significant values at ($p \leq 0.05$) as determined by one-way ANOVA with 'a' representing the minimum value.

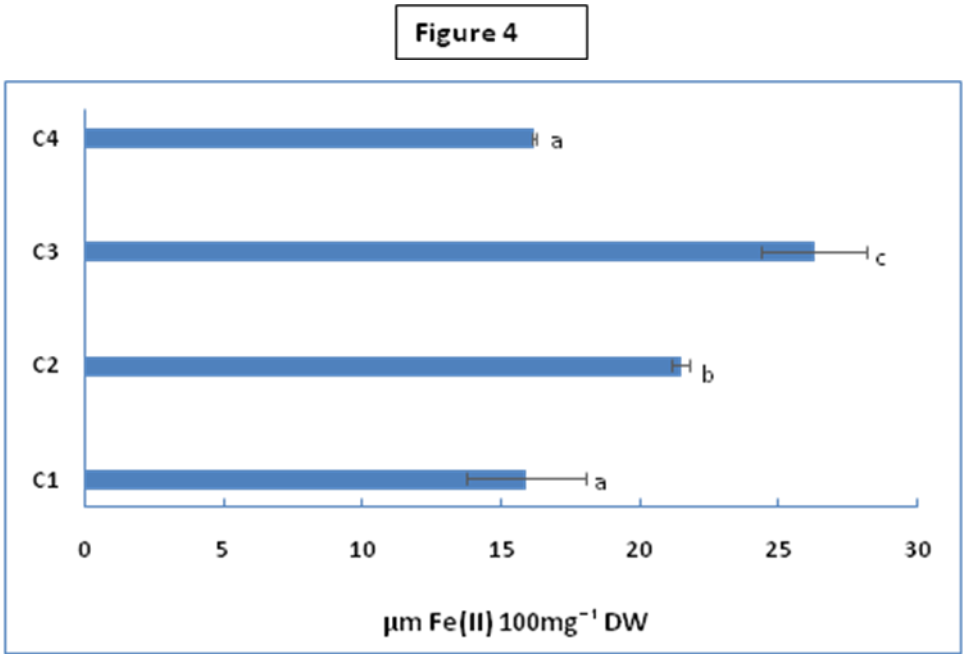


Figure 4. Chelating power activity (%) of *Cannabis* seeds from Jammu area. Different alphabet represents statistically significant values at ($p \leq 0.05$) as determined by one-way ANOVA with 'a' representing the minimum value.

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Conflict of interest

Authors declares no conflict of interest

Compliance with Ethical Standards

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors

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