# Nutritional value and antioxidant properties of *Cannabis* seeds from Makwanpur district of central Nepal

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**Abstract:** *Cannabis* seeds have been recognized as one of the most nutritionally complete food sources which are also used for medicinal as well as recreational purposes. In the present study, nutritional, phytochemical, antioxidant and antimicrobial properties of seeds of *Cannabis sativa* and *Cannabis indica*, collected from Makawanpur district, central Nepal were evaluated. Nutritional and phytochemical analysis were mainly based on the Association of Official Analytical Chemists (AOAC) methods, while antibacterial activity was tested using the agar well diffusion method. The results revealed that *Cannabis* seeds contain considerable amount of protein (32.08% to 43.04%), fat (30.86% to 42.40%), carbohydrate (8.39% to 13.79%), total phenolics (701.05 mg/100g to 1312.72 mg/100g), and total flavonoids (366.29 mg/100g to 385.12 mg/100g). The radical scavenging activity of the seeds ranges from 37.83% to 54.84% at the concentration of 6.25 µg/mL. The findings of our study indicate that both the species hold high nutritional contents and substantial antioxidant activities but could not exhibit antibacterial activity at tested concentrations.

**Keywords:** *Cannabis*; Seeds; Phytochemicals; Nutritional composition.

## Introduction

*Cannabis* belongs to the Cannabaceae family having three major species: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*, however, all three can also be called the subspecies of *Cannabis sativa*[1]. *C. sativa* and *C. indica* are believed to be originated from Eastern Asia, and the Hindu Kush Mountain range respectively[2]. They grow in wild and also cultivated in various parts of the globe including Nepal[3]. In south Asia, *Cannabis* species are cultivated for the production of marijuana, locally known as charesh. However, they are important sources of edible seeds, fibers and oil, and also used for medicinal and recreational purposes[4].They contain hundreds of compounds including cannabinoids and terpenes[5,6]. The two main constituents of these plants are cannabidiol (CBD) and tetrahydrocannabinol (THC). CBD is known to attune euphoric effects including antipsychotic, anticancer, antidiabetic, and neuroprotective effects[7]. With THC being the primary psychoactive constituent, these plants are highly regulated in the world. In recent times, some countries are relaxing laws against the consumption of *Cannabis* for both medicinal and recreational uses[8]. The capacity to endure drought and repel pests makes *C. sativa* seed production suitable even in harsh environments, and the seeds appeal as one of the most nutritionally complete food sources when used in the form of oil, flour, and protein powder[9]. *Cannabis* seeds are rich in unsaturated fats and

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protein, but have very little cholesterol[10]. It has been reported that 100 g of hemp seeds can meet up to 63 percent of the required daily protein for humans, and seeds are gradually making a comeback in many Western countries in the form of food and cosmetic products[10].

Due to the potential pharmaceutical and nutritional benefits, interest in hempseed is growing throughout the world in recent years. Canada, the United States, and Australia have made it legal to cultivate hempseed with low concentration tetrahydrocannabinol (<0.3 % w/w) [11]. The seeds are used in treating skin and subcutaneous tissue diseases and circulatory system disorders[12].

The use of the *Cannabis* seeds by the food industries as livestock feed supplements and as an ingredient for enriching or fortifying daily foods is also increasing[9]. Whole hemp seeds and hemp seed cake when included in the diets of animals have proven to be a valuable source of crude protein and essential fats showing a positive effect in the enrichment of animal products with n-3 polyunsaturated fatty acids and n-3 fatty acids[13]. Chickens fed with a hemp seed diet provided eggs with 10-20% more enriched yolk fat with α-linolenic acids, without any negative impact on laying performance[13]. Moreover, the seed extracts have inhibitory effects against various pathogenic microorganisms like Gram-positive and negative bacteria, viruses, and yeasts[14–16] and as an anti-biofilm mediator against *S. aureus*[17].

*Cannabis* seeds have long been a part of the local cuisine in Nepal and are roasted, cooked made into *Chutney* or eaten raw[18]. Although there are reports on the nutritional and phytochemical properties of *Cannabis* seeds from other parts of the world, little is known about the plants of Nepalese origin. It is very important to analyze local *Cannabis* seeds, because the nutritional, phytochemical composition, and bioactivities of the plants are linked to genotype, geography, climatic conditions, and local agronomic factors[19]. Therefore, the present study aimed to analyze the nutritional, phytochemical, antioxidant and antibacterial properties of the *Cannabis* seeds of Nepalese origin collected from Makawanpur district.

## Materials and methods

### Collection and identification of *Cannabis* species

*Cannabis* seeds were collected in February 2021 from several locations in the northwest corner of the Raksirang Rural Municipality, Makwanpur district ranging from 300 meters to over 2300 meters above sea level (Table 1). *Cannabis* species were identified by morphological observations of leaves, seeds, and flowers[20]. The matured *C. indica* seeds were larger than *C. sativa* seeds (Figure 1). The seeds were kept in zipper bags and transported to the Biological Resource Laboratory, Nepal Academy of Science and Technology (NAST). The species were re-confirmed with help of standard literature and herbarium specimens.

**Table 1. *C. indica* and *C. sativa* from different altitudes**

| Type | **Location** | **Sample code** | **Zone of collection** |
|---|---|---|---|
| ***C. indica*** | Raksirang-04, Jaisintal | IJ | Sub-tropical |
| ***C. indica*** | Raksirang-03, Piple | IP | Tropical |
| ***C. indica*** | Raksirang-04, Tarsikot | CI | Temperate |
| ***C. sativa*** | Raksirang-04, Jaisintal | SJ | Sub-tropical |
| ***C. sativa*** | Raksirang-04, Tarsikot | ST | Temperate |

**Preparation of working samples**

Healthy seeds were separated, washed with sterile water, and dried for a week in the shade before being oven-dried for 72 h at 40 °C. In the samples, bacterial and fungal contaminations were rigorously checked. Then, the samples were converted into fine powders with the help of a grinder and stored in air-tight plastic containers with suitable labeling and kept in dry storage.

**Macronutrients Determination**

**Protein content**

Protein was determined using a modified Bradford assay[21]. Two hundred milligrams of dried powdered sample was mixed with 20 mL water (Millipore, Milli-Q) and incubated in shaking incubator (Innovative Life Science Tools, USA) for 24 h at 50 °C and 100 RPM. After 24 h, the solution was filtered using Whatman no. 1 filter paper (GE Healthcare Limited, UK). The filtrate was used for the determination of protein content. In a 1:10 ratio, the sample filtrate and a freshly produced Bradford reagent were added to the 2 mL microfuge tube. The test was carried out in triplicates. All of the tubes were adequately vortexed and incubated for at least 5 min at room temperature. To minimize the effect of incubation duration on absorbance the samples were incubated at room temperature for no more than 1 h[22]. The absorbance of each 200 µL mixture was measured at 595 nm against the blank in a Multiskan Sky/Microtitre spectrophotometer ThermoFisher Scientific, USA) equipped with SkanIT software version 5.0. Bovine serum albumin (HIMEDIA Laboratories Private Ltd., Mumbai India) was used to calculate the standard curve (0-50 µg/mL; y= 0.002x + 0.143; $R^2$=0.992) and the results were expressed in g/100 g of sample.

**Carbohydrate content**

A colorimetric method using a modified anthrone reagent was used for this analysis[23]. Pretreatment of 0.5 g samples with 15 mL of 52% (v/v) $HClO_4$ and 10 mL of Milli-Q water was carried out and stored in the dark for 18 h[24]. After this period, samples were filtered, and the volume of the filtrate was diluted ten times. The volume of 5 mL of 0.1% (w/v) anthrone solution in 70% (v/v) $H_2SO_4$ was added to 1 mL of sample extract. The mixture was kept in a boiling water bath for 12 min where the anthrone reaction with sugars yielded a green color. Then, a cooled mixture (200 µL) was added to 96 well-plate, and the absorbance was measured at 630 nm in the microplate reader (Agilent Technology Cary UV-Vis). The absorbance of glucose was used to calculate the standard curve (0-80 µg/mL; y= 0.002x

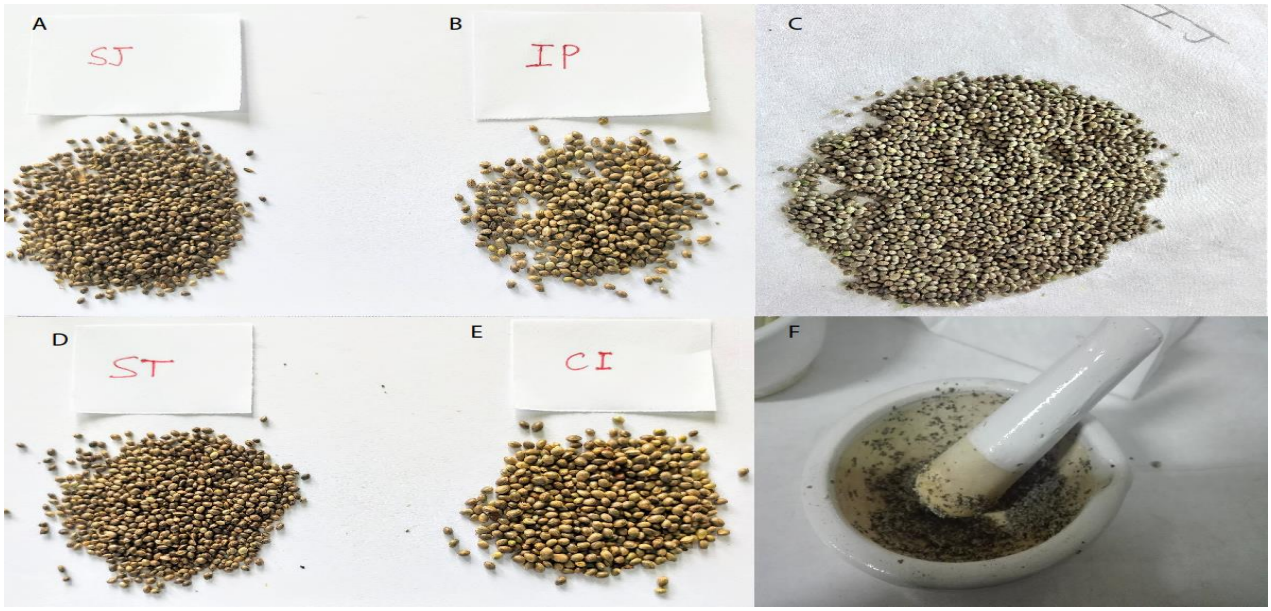


**Figure 1: Five different *Cannabis* seed samples. A and D: seeds of *C. sativa*. B, C and E: seeds of *C. indica*. F: Grinding of seeds before the extraction.**

+ 0.079; $R^2$ = 0.996) and the results were expressed in g/100 g of sample.

**Fat content**

The dry seed powder (5 g) was weighed on a glazed paper and transferred into an extraction thimble. The thimble was introduced into the Soxhlet extractor (SM Scientific Instruments, India) over a pad of cotton wool so that the top of the thimble was well above the top of the siphon. A clean, dry, round bottom flask was weighed and fitted with the extractor. Petroleum ether about 100 mL (HIMEDIA Laboratories Private Ltd., Mumbai, India) was poured along the side of the extractor until it began to siphon off[25]. The equipment was assembled with the flask at 40 °C, and the extractor was connected with the condenser. The cool water circulation was started in the condenser, and the extraction was allowed for 6 h. The thimble was removed with the material from the extractor. The ether was evaporated at 40 ºC to dryness using a rotary evaporator-HS-2005 V-N (Hahnshin scientific co., South Korea). The receiver flask was dried in a hot air oven at 100 ºC for 1 h, cooled, and weighed. The result was expressed as g/100 g of sample for fat content[30].

**Ash content**

Ash content was estimated using the dry ashing method[26,27]. After weighing 0.5 g of sample in a silica crucible, the sample was incinerated in a cold muffle furnace set at 550 ºC until whitish/grayish ash was obtained. The silica crucible was cooled, and the ash formed was weighed. The result was expressed as g/100 g of sample for ash content.

**Phytochemical analysis**

**Plant extracts preparation**

One gram of each dried and powdered *Cannabis* seeds were weighed separately, and 20 mL of methanol was added to both samples. The mixture was placed in a shaking incubator at 100 RPM at 37 ºC for 24 h. The mixture was filtered through Whatman no. 1 filter paper and the filtrates were stored at 4 ºC. With 20 mL methanol addition to the residue, the mixture was placed again in a shaking incubator at 100 RPM and 37 ºC for 24 h. Then, the mixtures were filtered through Whatman no. 1 filter paper. Finally, all the filtrates from the 1st and 2nd filtration were mixed. The samples were evaporated to dryness in a rotary evaporator at 40 ºC. The seed extracts were stored in a refrigerator until further analysis and they were re-dissolved in methanol accordingly when tests were performed.

**Total phenolic content**

Total phenolic content was estimated using a modified Folin-Ciocalteu assay[28]. To 50 µL of seed extract, 150 µL of Folin and Ciocalteu's phenol reagent (Merck Specialities Private Limited) was added. After 3 min, 150 µL of a saturated sodium carbonate solution was added to the mixture, and the volume was adjusted to 1500 µL with milli-Q water. The reaction was kept in the dark for 90 min. Then, 200 µL of the reaction was added to the 96 well-plate, and absorbance was read at 725 nm in the spectrophotometer. Gallic acid was used as a reference standard to calculate the standard curve (25-500 µg/mL; y = 0.0005x + 0.063; $R^2$ = 0.950). The result was expressed as mg of gallic acid equivalents (GAEs) per 100 g of extract.

**Total flavonoid content**

Total flavonoid content was estimated using the modified aluminum chloride-method[29]. To 100 µL of seed extract, 100 µL of 2% $AlCl_3.H_2O$ solution was added. The mixture was incubated in the dark for an hour, and absorbance was read in the spectrophotometer. All tests were performed in triplicate. The absorbance of Quercetin (Sigma-Aldrich) was measured to calculate the standard curve (25-250 µg/mL; y = 0.018x + 0.055; $R^2$ = 0.986). The result was expressed as mg of quercetin equivalents (QAEs) per 100 g of extract.

**Vitamin C content**

To determine vitamin C content, the modified Klein-Perry (1982) method was used[30]. For this, 50 mg dry methanolic extract of *Cannabis* seeds was extracted in 5 mL of 1% meta-phosphoric acid for 45 min at room temperature. The solution was filtered through Whatman No. 4 filter paper. To 100 µL of each sample, 900 µL of 2,6-dichlorophenolindophenol (HIMEDIA Laboratories

Private Ltd., Mumbai, India) was added and mixed properly. Then, 200 µL of reaction was added to 96 well plate, and the absorbance was taken within 30 min against a blank (methanol) was measured in the spectrophotometer at 515 nm. All tests were performed in triplicate. The absorbance of L-Ascorbic acid (CDH Laboratories) was determined to calculate the standard curve (25-100 µg/mL; $y = -0.002x + 0.692$; $R^2 = 0.981$). The result was expressed as mg of ascorbic acid/100 g of extract.

**β-carotene and lycopene content**

To determine β-carotene and lycopene content, the modified Nagata and Yamashita (1992) technique was used[31]. After weighing 100 mg of the dried methanolic extract, 10 mL of the acetone-hexane mixture (4:6) was added for 1 min. The mixtures were shaken vigorously and filtered through Whatman no. 4 filter paper. After that, 200 µL of the sample was added to the plate, and absorbance was measured at 453, 505, and 663 nm respectively in the spectrophotometer. All samples were assayed in triplicate. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/ 100 mL) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$ and β-carotene (mg/ 100 mL) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$. The results were expressed as µg of carotenoid/g of the sample.

**DPPH radical-scavenging activity (DPPH assay)**

The radical scavenging activity of the stable 1,1–diphenyl-2 picrylhydrazyl (DPPH) (Sigma-Aldrich) free radical activity was used to evaluate the antioxidant activity of the methanolic extracts of all samples according to the Blois method[32]. Ascorbic acid was used as control. Various concentrations of the seed extracts (100 µL) were mixed with 900 µL of a methanolic solution containing DPPH radicals ($6 \times 10^{-5}$ mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark. Then, 200 µL of each reaction mixture was added to the 96 well microtiter plate and the reduction of the DPPH radical was determined by measuring absorbance at 517 nm using the spectrophotometer (ThermoFisher Scientific, USA). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation % $RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where $A_S$ is the absorbance of the control when the sample extract has been added at a particular level and $A_{DPPH}$ is the absorbance of the DPPH solution.

**Antimicrobial assay**

**Crude extract preparation**

Five grams of powdered seeds was dissolved in 25 mL of ethanol for three days and filtered through Whatman filter paper no. 1. The filtrate was evaporated to dryness in a rotary evaporator at 40 °C. The extract was kept at 30 °C for a week to obtain a completely dry sample and stored at 4 °C for further analysis.

**Working sample preparation**

Different concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/mL) of working samples were prepared by dissolving dried seeds extract in 5% dimethyl sulfoxide (DMSO).

**Agar well diffusion test**

*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70062), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 19606), and *Staphylococcus aureus* (ATCC 25923) were cultured at 37 °C on nutrient agar. Mueller Hinton Agar (MHA) was used for the test and gentamicin was used as the positive control while 5% DMSO was employed as the negative control[33].

**Data analysis**

All the measurements were taken in triplicates except fat content and ash content and values were reported as mean±S.D. GraphPad prism 8.4.3 and MS-Excel 2007 were used for data analysis and graphical illustrations. Adobe Illustrator 2020 was used for preparing the illustration.

**Results**

**Nutritional analysis**

In a comparison of three *C. indica* samples, IJ had the highest protein content (43.04 ± 0.002 g/100g) and IP had

the lowest (32.82 ± 0.001 g/100g). Two samples of *C. sativa*, i. e. SJ and ST contained similar amount of protein (33.25 ± 0.005 g/100g and 32.08 ± 0.002g/100g), while CI revealed slightly higher value (36.76 ± 0.001g/100g). The carbohydrate content among all three samples (IJ, IP, and CI) is almost similar (13.79 ± 0.006, 12.92 ± 0.012, and 13.71 ± 0.006 g/100g respectively). IP contained the highest fat (36.84%) and the ash content (6.3%). The fat content was more in CI (35.71%) than in IJ (30.86%) while IJ contained more ash (6.22%) than CI (3.14%). The sample IJ contained the highest protein (13.79 ± 0.002) while CI contained the highest fat (42.4%), and the least ash content (3.14%). Figure 2 displays the nutritional composition among these 5 samples.

**Phytochemical analysis**

Among *C. indica*, the highest total phenolic content was found in IJ (1210.78 ± 0.03 GAE mg/100g), while the highest amount of total flavonoids and vitamin C was observed in (Table. 2). Within *C. sativa* samples, the total phenolic (1312.72 ± 0.04 GAE mg/100g) and flavonoid (372.34 ± 0.0.047 QAE mg/100g) contents were higher in ST as compared to SJ (1040.87 ± 0.007 GAE mg/100g and 358.72 ± 0.0.07 QAE mg/100g). Vitamin C content was similar between those samples (28.39 ± 0.02 to 28.73 ± 0.01 mg/100g). Sample IJ contained the highest concentration of lycopene (2.25 µg/g), while IP had the highest amount of β-carotene (211.78 µg/g). The low concentration of lycopene (0.20 µg/g & 0.22 µg/g) and β-carotene (35.45 µg/g and 39.69 µg/g) was observed in CI and ST respectively. β-carotene was not detected in sample IJ (Table 2).

**DPPH assay**

Radical scavenging activity of the samples ranged from 37.82% to 74.86% within the concentration range of 6.25 µg/mL to 50 µg/mL. *C. sativa* (ST) showed the highest scavenging activity at 50 µg/mL and other samples also revealed similar scavenging activity (Table 3).

**Antibacterial assay**

The methanolic extracts of *Cannabis* seeds with concentrations ranging from 1.5625 mg/mL to 200 mg/mL were unable to inhibit five different ATCC cultures of the selected pathogens as no antimicrobial properties were observed at tested concentrations.

**Discussion**

*Cannabis* seed is gaining research interest because of its fastest growth and utilization in commercial products

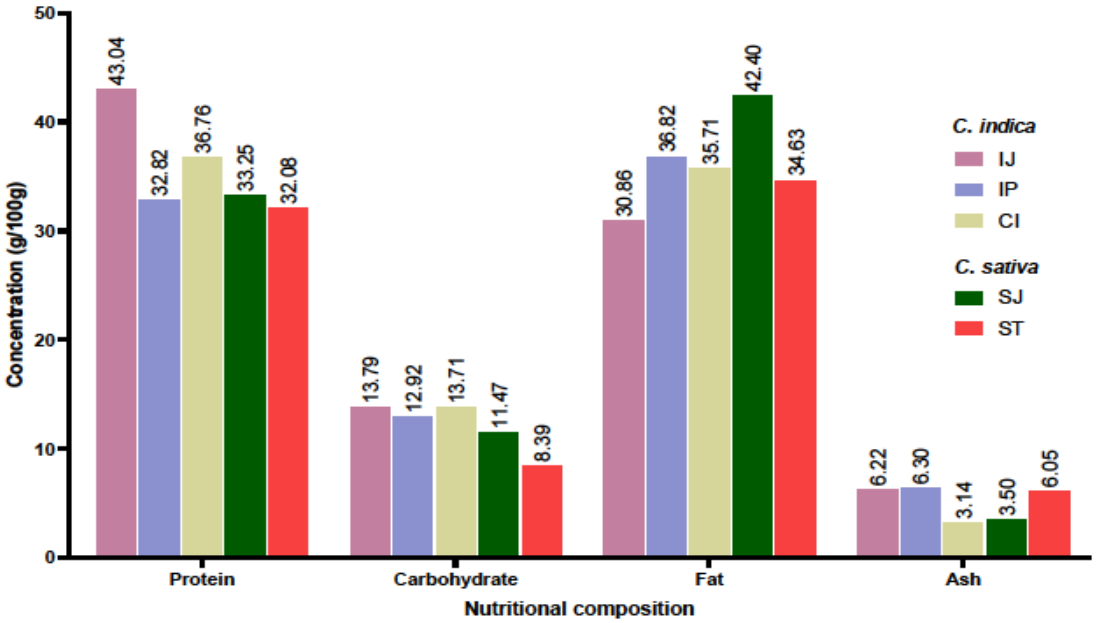


**Figure 2: Nutritional content in *C. indica* and *C. sativa* (g/100g ± S.D.)**

**Table 2. Phytochemical contents of *Cannabis* seeds**

| Sample code | Phenols (GAE mg/100g) | Flavonoids (QAE mg/100g) | Vitamin C (mg/100g) | Lycopene (µg/g) | β - carotene (µg/g) |
|---|---|---|---|---|---|
| *C. indica* | | | | | |
| **IJ** | 1210.78 ± 0.03 | 366.29 ± 0.09 | 20.70 ± 0.01 | 2.25 | ND |
| **IP** | 701.05 ± 0.02 | 371.79 ± 0.03 | 20.26 ± 0.005 | 0.62 | 211.78 |
| **CI** | 1142.81 ± 0.02 | 385.12 ± 0.02 | 21.02 ± 0.07 | 0.20 | 35.45 |
| *C. sativa* | | | | | |
| **SJ** | 1040.87 ± 0.07 | 358.72 ± 0.07 | 28.73 ± 0.01 | 0.42 | 56.83 |
| **ST** | 1312.72 ± 0.04 | 372.34 ± 0.05 | 28.39 ± 0.02 | 0.22 | 39.69 |

including textile, paper, medicine, food, animal feed, paint, biofuel, biodegradable plastic, and construction materials[31]. In this study, nutritional and phytochemical analyses of methanolic seed extracts of C. sativa and C. indica were presented based on nutritional contents like protein, carbohydrate, fat, ash, and phytochemical contents like phenols, flavonoids, vitamin C, lycopene, and β-carotene. In the present study, protein content of the seeds ranged from 32.08% to 33.25%, carbohydrate from 8.39 % to 11.47 %, fat from 34.63% to 42.40%, and ash from 3.50 to 6.05% among *C. sativa* samples. Similarly, in case of *C. indica* samples, protein content ranged from 32.82 to 43.04%, carbohydrate from 12.92% to 13.79%, fat content varied from 30.86% to 36.82% and ash content was found 3.14% to 6.22%. Some studies reported the protein content in the seed to be 11.60%[34] and 17.42%[15] indicating adequate protein content in our samples. The seed samples in the present study had significantly higher amount of protein but notably lower carbohydrate than previous reports[15,34]. Similarly, fat content of the seeds employed in the present investigation was higher than previous findings[34]. Ash contents reported by previous studies showed huge variations (1.90% and 12.74%)[15,34], while it ranged between 3.14-6.30% in the present study.

Such a variation in the nutritional composition of seeds might be due to the variation in geography, weather conditions, and local agronomic practices[19]. However, inadequate studies regarding the nutritional composition of *C. indica* contributed difficulty to compare our results with other studies. The presence of several classes of secondary metabolites and their varied concentrations in particular plant is often attributed towards their therapeutic potential. Phytochemical evaluation of *Cannabis* spp. in this study revealed the presence of phenols, flavonoids, β-carotene

**Table 3. RSA (%) of *Cannabis* seeds at different concentrations**

| Sample | Radical Scavenging Activity (RSA) (%) | | | |
|---|---|---|---|---|
| | 50 µg/mL | 25 µg/mL | 12.5 µg/mL | 6.25 µg/mL |
| *C. indica* | | | | |
| **IJ** | 73.70 | 56.81 | 63.61 | 54.71 |
| **IP** | 74.21 | 71.73 | 65.45 | 54.71 |
| **CI** | 73.04 | 66.10 | 57.72 | 37.83 |
| *C. sativa* | | | | |
| **SJ** | 73.30 | 69.11 | 60.86 | 40.31 |
| **ST** | 74.87 | 71.86 | 66.10 | 54.84 |

and lycopene, and vitamin C in varying quantities. Among the samples total phenols were found highest in *C. sativa* (ST), flavonoids in *C. indica* (CI), vitamin C in *C. indica* (SJ), lycopene, and β-carotene in *C. indica* (IJ and IP respectively). Both phenols and flavonoids have been reported for antiallergic, antiinflammatory, antimicrobial, anticarcinogenic, antimutagenic, and anticancer properties. Flavonoids are free radical scavengers that protect cells from oxidative damage when consumed in small amounts[35].

The total phenolic content in *Cannabis* seed extracts in our study varied from 701.05 mg/100g to 1312.72 mg/100g. High total phenol content (1419 mg/100g) was reported on *Cannabis* seeds from Nigeria[36]. However, very low phenolic content, that ranged between 6.70 to 12.87 mg/100g was found in Carmagnola variety of *Cannabis* seed extract[37]. On the other hand, flavonoid content in the present study ranged between 366.29 mg/100g to 385.12 mg/100g, however a previous study reported 1266 mg/100g[36], which is quite higher than our results.

Vitamin C is a water-soluble nutrient that is required for life due to its involvement in numerous metabolic activities and enzymatic reactions. It is necessary for the formation of cartilage, teeth, and bones, as well as the healing of wounds and the absorption of iron from the intestine[38]. In our study, the content of vitamin C ranged from 20.70 to 28.73 mg/100g. Carotenoids such as β-carotene and lycopene, as well as some of their metabolites, are thought to play a protective role in a variety of reactive oxygen species (ROS) mediated disorders, including cardiovascular disease, cancer, neurological, hepatic, and reproductive disorders, diabetes mellitus, and photosensitive or eye-related disorders[39,40].

The β-carotene in *Cannabis* seed extracts ranged from undetectable amount to 211.784 µg/g whereas lycopene concentrations were between 0.20 µg/g to 2.25 µg/g in our investigation. The recent values were lower than previous studies which reported much higher β-carotene and lycopene levels i.e. 2410 µg/g and 1880 µg/g respectively[36]. Similar variation in β-carotene and lycopene content was also reported in other plant sources. For example, β-carotene content of yellow and red colored watermelon was reported to be 0.05 µg/g and 6.16 µg/g respectively by Charoensiri et. al., (2009) while lycopene was undetectable and 66.93 µg/g respectively[41]. In another study Setiawan et. al., (2001) estimated lycopene and β-carotene to be 1.4 µg/g and 0.71 µg/g respectively in the yellow variety while the amount of lycopene and β-carotene were 5.92 µg/g and 113.89 µg/g respectively in the red variety[42].

The extract was more able to scavenge DPPH radicals and chelate ferrous ions[41]. At 50 µg/mL, *C. sativa* (ST) had the highest scavenging activity, although all of the samples, including ST, had similar scavenging activity. Essential oils extracted from legal hemp varieties (THC <0.2% w/v) showed some inhibitory activity against microbes like food-borne pathogens, gastrointestinal bacteria, and yeasts[44]. None of the extracts showed a zone of inhibition against the bacterial strains used in our study.

The concentration of seed extracts in our study was not sufficient to show antibacterial activity so usage of a higher concentration is recommended for future analysis since instead of oil; the methanolic extract from the whole seed was used in our study. The gene expression involved in the manufacture of secondary metabolites in medicinal plants is believed to be influenced by the environmental factors. The content and amount of those secondary metabolites may be affected by genetic and abiotic environmental factors such as geographic features (latitude, altitude), light, climatic, edaphic, and biotic interactions[45].

**Conclusion**

*Cannabis* seeds analyzed in this present study showed a range of nutritional composition and phytochemicals. The seeds of *C. sativa* and *C. indica* contained a high amount of protein which may be a potential source of protein supplement. Higher content of fat showed that the seeds could be used for oil extraction. Similarly, the greater amount of total phenolics coupled with reasonable antioxidant activity signifies towards their antioxidant

potential. However, the methanolic extract of both varieties of *Cannabis* seeds did not show antibacterial property at given concentration.


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