Agriways 7 (2) : 87-95 December, 2019

ISSN: 2321-8614 (Print)
ISSN: 2454-2318 (Online)

Research Article

SCREENING SPROUTS OF GREEN MUNG (*VIGNA RADIATA*) AND CHICKPEA (*CICER ARIETINUM*) FOR BIOACTIVE COMPOUNDS AND PROTEASE ACTIVITY BY USING VARIOUS METHODS AND NMR SPECTROSCOPY

Bhawanpreet Kaur[1], Sukhwinder Kaur[2] and Nitu Trehan[2]
[1] Department of Biotechnology, Mata Gujri College, Fatehgarh sahib
[2]Department of Microbiology, Mata Gujri College, Fatehgarh sahib
Email: bhawanpreet01@gmail.com

Received: 22 Sept 2019/Accepted: 12 Dec 2019

Abstract The aim of the study is to access the screening sprouts of Green gram (*Vigna radiate* L.) and Chickpea (*Cicer arietinum* L.) for Bioactive compounds and Protease activity by using various methods and NMR Spectroscopy. The sprouted seeds of green mung and chickpea were powdered and was extracted with Tris HCl buffer. Protein content and protease activity were evaluated using Lowry method and Casein assay. For bioactive compounds, the antioxidant activity was done by DPPH (2,2-diphenyl-1-picrylhydrazyl) method, Total flavonoid content (TF) and Total Phenolic content (TP) were determined by Aluminium chloride, Folin-ciocelteu assay. Characterization and NMR spectroscopy of green gram and chickpea were analyzed. Sprouting increased the protein concentration, Total flavonoid content (TF) and antioxidant activity in green gram (0.7 mg/g), (0.00035 mg/g), (47.5%) than chickpea was (0.35 mg/g), (0.00011 mg/g), (33.6%). Protease activity accounted highest in chickpea. Green gram showed maximum catalytic activity was at 37°C and at pH 6 whereas chickpea was at 37 to 40° C, at pH 8. Total phenolic content (TP) was more in chickpea (0.0471 mg/g) and less in green gram (0.0305 mg/g).The Nuclear magnetic resonance (NMR) analysis of sprouted seeds elaborated the existence of saccharides, fatty acids, amino acids, phenolics, bioactive compounds that is used in pharmaceutical industries for research and development for active pharmaceuticals ingredients, drugs and other formulations.Thus, biochemical analysis and their characterization proven importance of sprouted seeds in our daily life. Overall green gram is an important pulse crop with a diverse array of potential nutritional and health benefits.
Keywords: Sprouts, highest, leguminous, mung bean, chickpea, activity, diet and source

Introduction

Plants which provide pulses belonging to the family Leguminosae that have been used as food for thousand years also known as legumes; they are abundant in proteins and are easily digestible (Munoz *et al*., 1996). Germination initiates large number of enzymes mainly protease that remove or slow down antinutritional and indigestible factors thereby improving the quality of legumes by enhancing its nutritive value and also increases the level of carbohydrates, fatty acids, vitamins, minerals such as calcium, magnesium, zinc, iron, potassium and phosphorus and phytonutrients naturally (Bau *et al.,* 1997). In addition, health promoting factors some antinutritional properties of plant phenolics are of great importance to consumers (Shahidi *et al.,* 2004). Proteases are present in viruses, archaea, bacteria, plants and animals (Veloorvalappil *et al*., 2013). Peptidases play crucial role in digestion, photosynthesis, apoptosis (programmed cell death), viral pathogenesis, processing, regulation of proteins and many more. Depending upon the mechanism of action, proteases are classified as serine, cysteine, threonine (amino-terminal nucleophile hydrolyses), aspartic and glutamic proteases. They are currently used to a great extent in the pharmaceutical, leather and various foods processing industries. Sprouts are heart friendly, inexpensive, source of fat free diet (reduce obesity) which boosts the immune system and brain functioning. *Vigna radiata* commonly known as mung bean / green gram / green mung and belongs to family Leguminosae. Green gram sprouts are the potential source of vitamins A, B, C and E, minerals, iron, potassium, calcium, magnesium, phytochemicals, proteins and amino acids. The seeds and sprouts are the excellent examples of functional foods and prevent the risk of various diseases (Pasko *et al*., 2008). It enhances the enzyme activity which is necessary for food digestion. *Cicer arietinum* commonly known as chickpea

/ garbanzo beans and it also comes under Leguminosae family. These sprouts also have a lot of vitamins, iron, calcium and magnesium. Chickpea is cholesterol free and contains good source of dietary fibers and minerals (Wood *et al.,* 2007). It can be used as salads, soups or fried or steamed with other bean sprouts and vegetables. This study was to investigate the effect of germination on biochemical composition and protease content in mung beans and chickpea sprouts. Chickpea has been used to treat protein malnutrition and Kwashiorkor in children (Krishna *et al.,* 1975).

Method and Materials

2.1 Isolation and preparation of protease enzyme (crude extract)

The seeds of green mung and chickpea were purchased from local market located in Khanna. The seeds were not chemically treated in any way as this would slow down germination rate. For sprouting process, seeds were washed thoroughly in tap water and then soaked overnight in fresh water for 8-10 hours. They were allowed to germinate at least for three to four days the tail like protrusion from seeds i.e.; sprouts used for further experiments. Both the sprouted seeds were homogenates, finely powdered in a pre-chilled mortar and mixed with chilled 10mM Tris-HCl buffer at pH 8.0 containing 2M NaCl for 3 hours. The extracted mixtures were filtered through gauge and filtrates were centrifuged at 10,000 rpm for 10 minutes below 4°C. The collected supernatant used for the estimation of extracellular concentration of protein and further biochemical analysis.

2.2 Biochemical analysis:

2.2.1 Protein estimation by Lowry method (Lowry *et al.*, 1951)

Protein concentration was determined by the method using bovine serum albumin (BSA) as standard protein. The amount of the soluble protein was calculated from the standard curve as mg of protein per ml of test samples. The different concentrations of stock solution of protein were made i.e. BSA ranging from 0.1 to 1 ml. Added 4 ml of alkaline copper reagent in each tube, stirred them and incubated at room temperature for 10 minutes. Similarly, added 4 ml of Folin-ciocalteau reagent to all the test tubes and incubated at room temperature for 30 minutes. After incubation absorbance was taken at 660 nm or 720 nm.

2.2.2 Determination of Protease activity by Casein hydrolysis or Tyrosine assay (Anson *et al.,* 1938)

The proteolytic activity of extract was analyzed against casein in order to record the activity of protease. A protease unit is defined as the amount of enzyme that releases 0.1 micromole of tyrosine per ml per min under assay conditions. The reaction mixture consisted of 1ml extract (crude enzyme) and 5ml of casein as a substrate for alkaline pH was incubated at 37^0C for 30 minutes. The reaction was terminated by the addition of 10% trichloroacetic acid (TCA). For control, the substrate was precipitated with 10% TCA before adding the extract and treated same as described above. The precipitated comes out were removed by centrifugation and supernatant was added to alkaline solution and allowed to stand for 30 minutes. Then the mixture was again mixed well with Folin-ciocalteau reagent. The absorbance was measured at 660 nm by spectrophotometer. The protease activity was expressed as difference between control and test sample.

2.2.2.1 Characterization of Protease enzyme:

Effect of temperature and pH on protease activity.

Optimum temperature and pH of protease activity were determined using various temperatures (10°C, 20°C, 30°C, 40°C, 50°C, 60°C) and pH (2.0, 4.0, 6.0, 8.0, 10.0, 12.0).

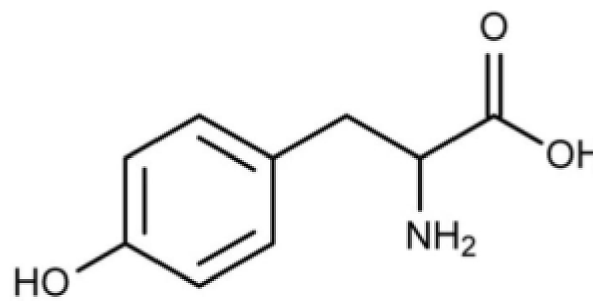

Figure 1 Structure of Tyrosine

2.3 Estimation of Bioactive compounds and antioxidant activity.

2.3.1 Determination of Total Flavonoid Content (TF) (Zhu *et al.,* 2009)

Total flavonoid content was determined by the aluminium chloride spectrophotometric method. In this method, Quercetin was used as standard and flavonoid contents were measured as quercetin equivalents. The standard curve of quercetin was prepared 1 ml of standard or extract solution (10, 20, 30, 40, 50, 60, 70, 80, 90, 100

mg/l) with 4 ml of distilled water, then added 0. 3 ml of 5% $NaNO_2$ to the flask after 5 minutes, 0.3 ml of 10% $AlCl_3$ was added to the mixture. At the 6th minute added 2 ml of 1M NaOH and volume was made up to 10 ml with distilled water. The absorbance was measured at 510 nm using spectrophotometer.

Figure 2 Structure of Quercetin molecule

2.3.2 Determination of Total Phenolic Content (TP) (Pourmorad *et al.*, 2006)

The amount of total phenolics in extracts was determined using the Folin-ciocelteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). For the standard concentration of 10, 20, 30, 40, 50, 60 mg/l of gallic acid were prepared in methanol. Concentration of 1000 ppm of extract was also prepared in methanol by added 0.5 ml of extract and mixed it with 2.5 ml of Folin-ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenol, thus producing a blue colour upon reaction. The absorbance was measured at 760 nm by using spectrophotometer.

2.3.3 Determination of antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Williams *et al.*, 1995)

Antioxidant activity was determined by DPPH method. This method is based on reduction of stable free radicals. For this assay, added 1ml of extract in each test tube after that 5ml of 0.004 % DPPH added in it, incubated at room temperature for 30 minutes. The absorbance was measured at 517 nm.

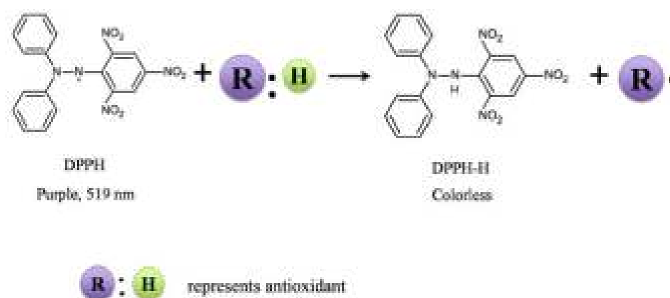

Figure 3 Showing the reaction by DPPH method.

Calculations:

Reduction % in DPPH is: % Reduction = (A0-A1)/A0] x 100

Where: A0 is absorbance of standard and A1 is absorbance of sample.

2.4 NMR spectroscopy analysis

All the prepared samples were tested on a JNM-ECS 400 NMR spectrometer (Bruke , Rheinstetten ,Germany) at a frequency of 400 MHz via a triple inverse gradient probe as NMR probe, with a temperature 25℃ (Chen *et al.,* 2019). All the samples were dissolved in DMSO-d6 (dimethylsulphoxide), vortexed and centrifuged. The supernatant was transferred to NMR tube. NMR spectral data was recorded at 25℃ and 400 MHz on a Bruker Avance spectrophotometer using H1. The spectra were obtained in 4 minutes per sample.

3 Results and Discussions

3.1 Yield of extract

The yield of extract in green gram was 13.88% and whereas in chickpea was 10.86%. Finally, green gram has higher yield of extract.

3.2 Biochemical analysis:

Identification of sprouts having high protease activity and biochemical potential is very beneficial information. The conformation of various test was employed to take the physical measurements and to quantify the observed values. The following methods were adopted to quantify the several constituents present in them.

3.2.1 Estimation of Protein Concentration by Lowry method

The standard plot of different protein concentration was drawn with BSA (Bovine serum albumin) from standard calibration plot, the protein concentration was estimated in terms of mg/g as shown in figure 4.

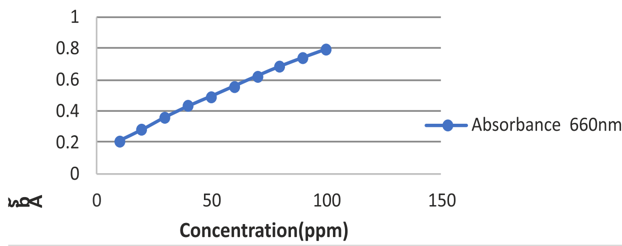

Figure 4 The standard calibration curve of BSA

Sprouted green gram has highest concentration of protein 0.7 mg/g whereas chickpea has least 0.35 mg/g. The results showed protein concentration was higher in green gram (Anwar *et al*., 2007). due to germination there was increase in nitrogen content, health promoting factors, fibers and many more. In the Nutshell, green gram shows higher nutritional value, short life cycle and easy availability is responsible for a good source of healthy diet.

3.2.2 Determination of protease activity by Casein hydrolysis or Tyrosine assay

The activity of protease was calculated from the standard tyrosine plot. The different concentrations of tyrosine were prepared on hydrolysis chickpea was found to be higher protease activity i.e.; 0.832 mg and green gram was 0.141 mg. The chickpea shows more protease activity than green gram.

Comparison of protein content and protease activity

Figure 5 shows the comparison view of protein content and protease activity in green gram and chickpea. Green gram was extremely proteinaceous in nature and chickpea contains more protease activity. Hence, green gram is protein rich and chickpea has more power of hydrolysis.

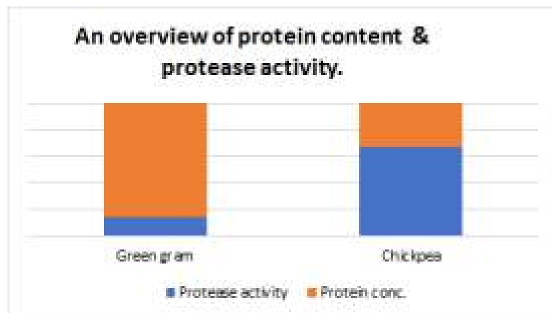

Figure 5 A Brief about the protein conc. and protease activity.

3.2.2.1 Temperature

(a) Effect of temperature on protease extracted from Green gram.

The stability of protease extracted from green gram at different temperatures was shown in figure 6. Optimum temperature of green gram was at 37°C beyond this range the protease activity starts decreasing due to denaturation of enzyme (Dahot *et al.,*1998). Consequently, the proteases isolated from this study marked as an excellent source of enzymes for industrial purposes and food industries which required mild temperatures as a vital factor in the processing steps.

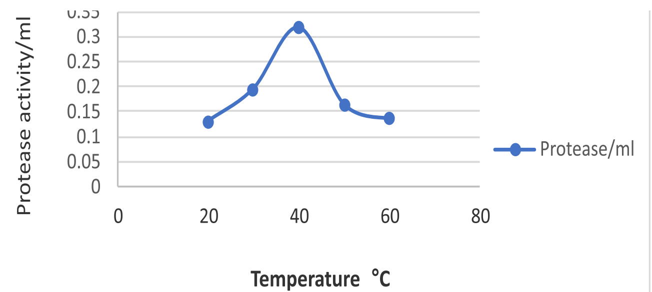

Figure 6 Effect of temperature on green gram

(b) Effect of temperature on protease extracted from chickpea.

Chickpea has higher catalytic efficiency at 40°C. At this range it shows maximum activity. The optimum range of temperature was between 37°C to 40°C (Shamshi *et al.,*2016*).* as shown in figure 7.

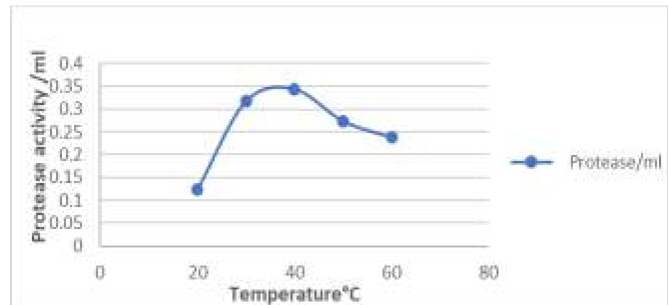

Figure 7 Effect of temperature on chickpea.

3.2.2.2 pH

(a)Effect of pH on protease extracted from Green gram.

Green gram shows maximum catalytic activity at pH 6 as shown in figure 8. At above or below range the catalytic

activity decreased. The observed result indicates the alkaline proteases involved in all seeds were seem to be more potent than the acidic proteases. Thus, alkaline protease plays an important role in industrial applications (Egwin *et al.,* 2011).

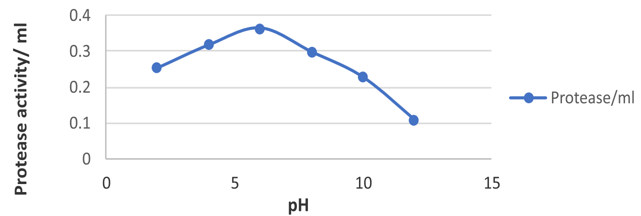

Figure 8 Effect of pH on green gram.

(b) Effect of pH on protease extracted from Chickpea.

The depicted graph concluded that the optimum pH of chickpea was found to be 8 i.e.; alkaline range as shown in figure 9. At pH it shows maximum catalytic activity. The optimum pH range of chickpea was found to be 8 (Shamshi *et al.,* 2016).

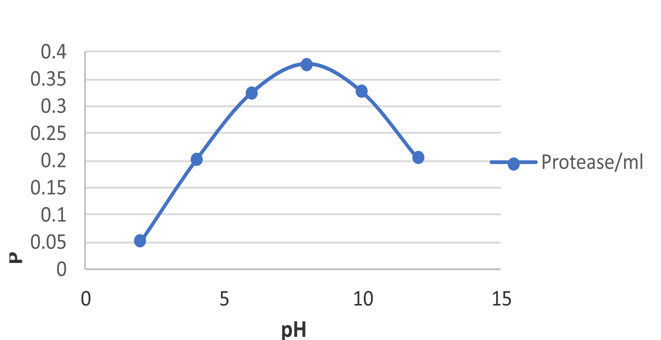

Figure 9 Effect on pH on chickpea.

3.3 Estimation of Bioactive compounds and antioxidant activity.

3.3.1 Determination of Total Flavonoid Content. (TF)

The standard plot of quercetin was shown in figure 10 from this data the flavonoid content in a sample was estimated.

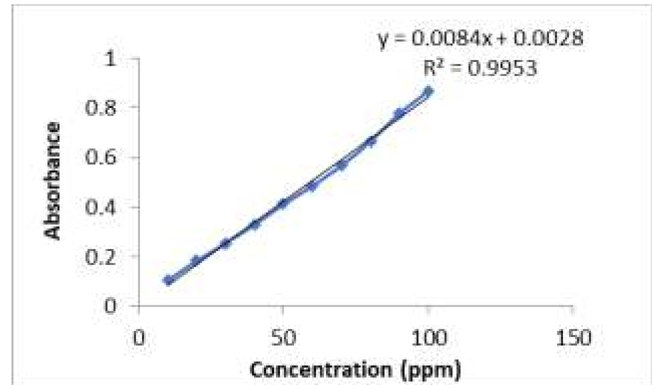

Figure 10 The standard plot of quercetin

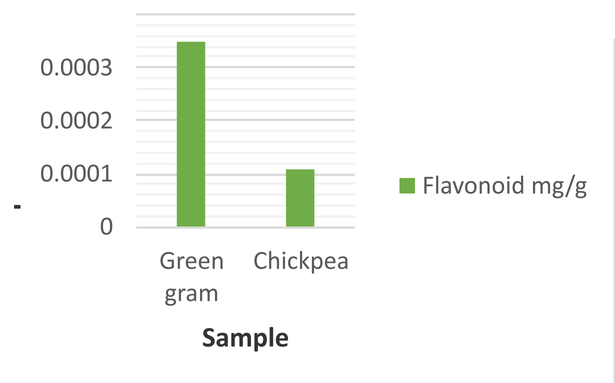

Figure 11 Total flavonoid content (TF) in green gram and chickpea

Total flavonoid content (TF) were expressed as mg/g quercetin equivalent. Total flavonoid content in chickpea was 0.00011 mg/g and in green gram was 0.00035 mg/g as shown in figure 11. Sprouted green gram has more flavonoids than chickpea as it causes natural changes in their metabolic properties. Vitexin and isovitexin are common flavonoids present during germination. Such compounds start rising due to their presence in outer seed coat. (Chon *et al.,* 2013). Hence germination increases flavonoids.

3.3.2 Determination of Total Phenolic Content (TP)

Gallic acid was used for standard calibration curve as shown in figure 12 from the compositional data total phenolic content in sample was estimated in terms of mg/g.

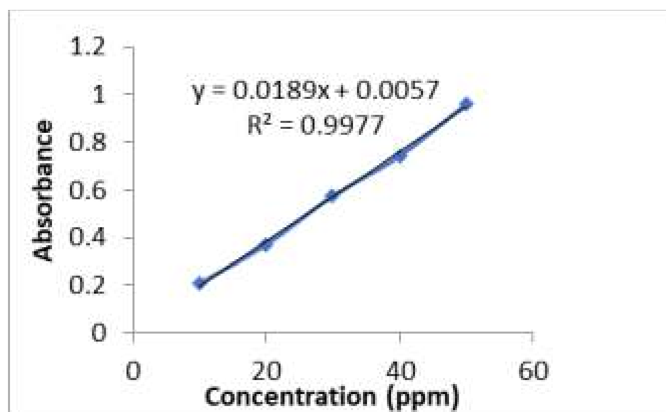

Figure 12 The Standard calibration curve of Gallic acid.

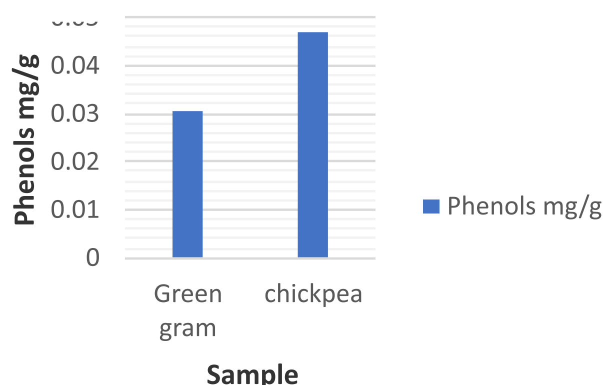

Figure 13 Total phenolic content (TP) in green gram and chickpea.

Total phenolic content (TP) in chickpea was 0.0471 mg/g and in green gram was 0.0305 mg/g as shown in figure 13. chickpea has more phenolics than green gram. The rise in polyphenolic content after germination has been broadly reported in chickpea. (Khattak *et al*., 2007). Total phenolic content (TP) depends on the extracting solvent, i.e. the polarity of solvent used in extraction. Due to the presence of water, their metabolism starts increasing, particles inside the grains change thus it generates large amount of energy and several new compounds (Lopez *et al.,* 2006). Sinapic acid and Cinnamic acid are common phenols found in plants (Proestos *et al*., 2013).

3.3.3 Determination of Antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) Method

Higher the % DPPH reduction, more is its antioxidant activity and lower the optical density, greater is its antioxidant power. From the above calculated data, green gram shows higher % DPPH activity 47.5% whereas chickpea shows lesser 33.6 % (Guo *et al.,* 2012). reported that the antioxidant activity of mung bean sprouts was 6 times higher than dry seeds. Legume germination has also been suggested as a powerful strategy to increase total antioxidant activity (Fernandz *et al.,* 2006).

Comparison of Bioactive compounds:

Bioactive compounds containing total phenolic content (TP), total flavonoid content (TF), and antioxidant activity (DPPH) in green gram and chickpea was summarized as shown figure 14.

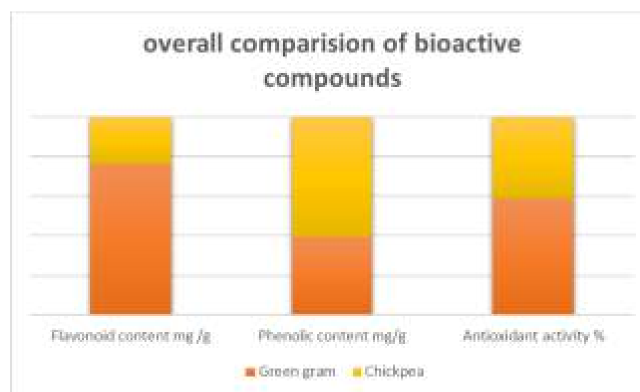

Figure 14 Comparative study of Bioactive compounds.

On comparison the bioactive compounds in sprouted green gram and chickpea concluded that green gram act as a good source of flavonoids and antioxidants. It enhances the metabolic rate and also helps to fight against diseases.

3.4 Generation of NMR database.

The resulting NMR spectrum of each sample was recorded manually for the phase and baseline distortion. The different compounds corresponding to peak were identified by H1 NMR spectra H1 Chemical shifts (ppm) used with reference to DMSO-d6 (Dimethylsulphoxide). Based on the chemical shifts of H1 NMR spectra, the representative in green gram and chickpea were analyzed.

NMR data of Green gram.

The NMR spectra of Green gram containing various compounds was detected as shown in figure 15 it elaborates the presence of different compounds corresponding to peaks were determined and the constituents detected was described in tabular form in table 2.

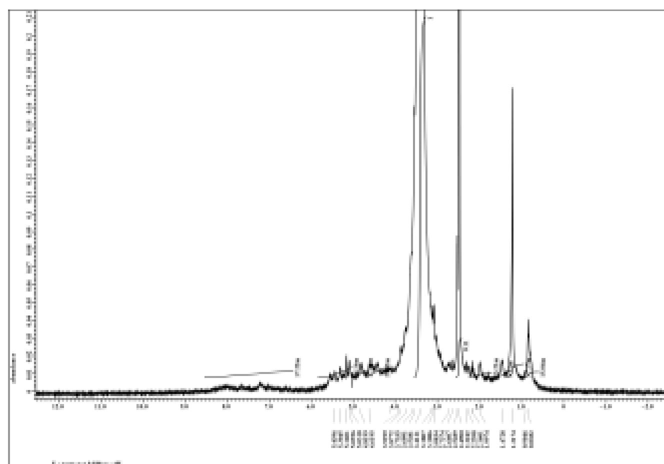

Figure 15 NMR spectra of green gram

The analysis of Green gram extract identified the presence of different components and the recorded quantification results as shown in table 2.

Table 2 Representative data showing the constituents present in Green gram.

ppm	Constituents
0.3-0.5	Monosaccharides
5.3	Unsaturated fatty acids
0.5-8	Amino acids
7.32	Phenylalanine
5.5-8.5	Bioactive compounds

The results presented the different detections of essential constituents in green gram sprouts. Multiple H1 signals were detected from 0.5 to 8 ppm. Moreover, most signals were recorded in the region of 0.5 to 8 ppm. Some additional signals were obtained in the region around 5.5 to 8 ppm, small signals were analyzed in the range of 0.3 to 0.5 ppm which established the presence of carbohydrates. Also, the presence of various chemicals containing carbohydrates, amino acids, bioactive compounds, phenolics, organic acids, fatty acids. The results were consistent with that of study (Liu *et al.* ,2019).

NMR data of Chickpea.

The NMR spectra of chickpea containing various compounds was detected as shown in figure 16 It shows the presence of different compounds corresponding to peaks were determined and the constituents detected was described in tabular form as shown in table 3.

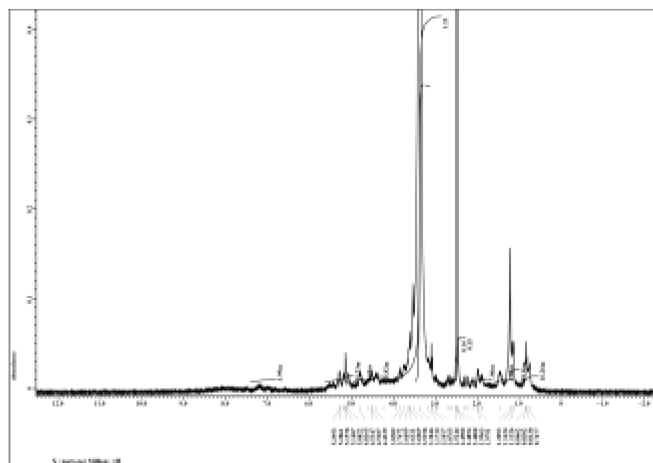

Figure 16 NMR spectra of Chickpea

The analysis of Chickpea extract identified the presence of different components and the recorded quantification results as shown in table 3.

Table 3 Representative data showing the constituents present in chickpea.

ppm	Constituents
0.5 to 5	Carbohydrates
1.5	Amino acids
3	Aspartic acid
7.4	Bioactive compounds

The results presented the different detections of essential constituents in chickpea sprouts. Multiple H1 signals were detected from 0.5 to 5 ppm. Moreover, most signals were recorded in the region of 1.5 ppm which established the presence of amino acids. Some additional signals were obtained in the region around 5.5 to 8 ppm, small signals were analyzed in the range of 0.3 to 0.5 ppm which established the presence of carbohydrates.

Discussion

In the present study showed metabolic changes occurred during sprouting of green mung and chickpea. Green mung is rich in protein, phenolics and good antioxidant power. The results of H1 spectral analysis evaluate the presence of different compounds in green gram and chickpea. The identification of amino acids in the spectra reveals its significance in amino acid translation events occurred in physiological processes

(Moore *et al*., 2016). The peaks beyond 5.5ppm (aromatic region) indicates the synthesis of phenolics during sprouting (Yuan *et al.,* 2017). In the plants, phenolics are significant to balance the redox equilibrium and helps to withstand the action of environment stress. The presence of fatty acids and carbohydrates were detected which helps to maintain the sugar metabolism and also responds to energy starvation process. Therefore, the overall study helps to clarify the importance of sprouting in our life and gives a reference for producing high quality products.

Conclusion

The results of this study showed that sprouted green mung and chickpea have been consumed in diet worldwide as it contains vital chemical constituents that plays a chief role in human nutrition especially as rich source of protein. Besides these, green gram possesses carbohydrates, fatty acids, flavonoids, antioxidants and their majorly role in health promoting factors, protection against degenerative disorders. It gives evidence for the health benefits of direct green gram consumption. They have great potential in food and industrial applications. Therefore, it proved the effectiveness of green gram in preventing and treating several symptoms and pathologies.

References

Anson ML. 1938. The Estimation of pepsin, Tripsin, Papain and cathepsin with Hemoglobin. *Journal of General Physiology* **22**: 79-89.

Anwar S, Latif R, Przybylskl B, Sultana and Ashrsf N. 2007. Chemical Composition and Antioxidant activity of Seeds of Different Cultivars of Mung bean. *Journal of Food Science* **72**:503-510.

Bau HM, Villanme C, Nicolos, JP and Mejean L. 1997. Effect of germination on chemical composition, biochemical constitutes and antinutritional factors of soybean (*Glycine max*) seeds. *Journal of Science Food Agriculture* **3**: 1-9.

Chen L, Wu JE, Zhanming L, Liu Q, Zhao X, Yang H. 2019. Metabolic analysis of energy regulated germination and sprouting of organic mung bean (*Vigna radiata*) using NMR spectroscopy. *Journal of Food Chemistry* **286**:87-97.

Chon SU. 2013. Total phenolics and bioactivity of seeds and sprouts of several legumes. *Current Pharmaceutical Design* **19**:6112-6124.

Dahot MU. 1992. Investigation of Proteases in plant seeds. *Journal of Islamic Academy of Sciences* **5**: 241-244.

Egwin EC. 2011. Partial characterization of protease from *Rhynchophorous palmarum* Palm weevil. *African Journal of Food Science Technology*. **2:**140-145.

Fernandez-Orozco R, Piskula MK, Zielinski H, Kozlowska H, Frias J and Vidal-Valverde C. 2006. Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. Zapaton. *European Food Research and Technology* **223**: 495-502.

Guo X, Li T, Tang K and Liu RH. 2012. Effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts (*Vigna radiata*). *Journal of Agricultural and Food Chemistry* **60**: 11050-11055.

Khattak ABA, Zeb N, Bibi SA and Khalil MS. 2007. Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts. *Journal of food Chemistry* **104**:1074-1079.

Krishna M, C.R 1975. Biochemical studies on Bengal gram. *Journal of Scientific and Industrial Research* **34**:266-281.

Liu Q, Wu JE, Lim ZY, Aggarwal A, Yang H and Wang S.2017. Evaluation of metabolic response of *Escherichia coli* to electrolysed water by H1 NMR spectroscopy. LWT- *Food Science and Technology* **79**:428-436.

Lopez-Amoros ML, Hernández T and Estrella I. 2007. Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal Food Composition and Analysis* **19**: 277-283.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**: 265-275.

Moore M, Gossmann N, and Dietz K.J. 2016. Redox regulation of cytosolic translation in plants. *Trends in Plant Science* **21**:388–397.

Munoz GE, Barlow PW and Palma B. 1996. Effects of sea water on roots of *Prosopis alba* (Leguminosae) seedling. *Phyton Buenos Aires* **59**: 55-63.

Proestos C and Komaitis M. 2013. Analysis of naturally occurring phenolic compounds in aromatic plants by RP-HPLC coupled to diode array detector (DAD)

and GC-MS after silylation. *Foods* **2**: 90-99.

Pourmorad F, Hosseinimehr SJ and Shahabimaid N. 2006. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. *African Journal of Biotechnology* **5**:1142-1145.

Pasko P, Sajewicz M, Gorinstein S, Zachwieja Z. 2008. Analysis of the selected phenolic acids and flavonoids in *Amaranthus ceuentus* and *Chenopodium quinoa* seeds and sprouts by HPLC method. *Acta Chromatographica* **20**:661-672.

Shamsi TZ, Sen P and Fatima S. 2016. Purification and Characterization of a Protease from Green Seeded Chickpea (*Cicer arietinum*). *Journal of Research and Development* **2**:100-146.

Shahidi F, Naczk M.2004. Phenolics in food and nutraceuticals: Sources applications and health effects. CRC Press Boca Raton, Florida.

Veloorvalappil NJ, Robinson BS, Selavanesan P, Sasidharan S,Kizhakkepawothail NU, Sreedharan S, Peaksan P, Moolakkariyil SJ,Sailas B 2013. Versality of microbial proteases. *Advance Enzyme Research* **3**:39-51.

Williams W, Cuvelier ME and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-*Food Science and Technology* **28**: 25-30.

Wood JA and Grusak MA. 2007. Nutritional value of chickpea. In chickpea breeding and management. *CAB International*, Wallingford **12**: 101-142.

Yuan Y, Zhao Y, Yang J, Jiang Y, Lu F, Jia Y, and Yang B. 2017. Metabolomic analyses of banana during postharvest senescence by 1H-high resolution-NMR. *Food Chemistry* **218**: 406–412.

Zhu H. 2009. Analysis of Flavonoids in *Portulaca oleracea* L. by UV-Vis Spectrophotometry with comparative study on different extraction technologies. *Food Analysis Methods* **3**: 90-97.