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Udita Tiwari

Department of Biochemistry,
School of Life Sciences, Dr. B.R.
Ambedkar University, Agra, Uttar
Pradesh, India

Ankit Servan

Department of Biochemistry,
School of Life Sciences, Dr. B.R.
Ambedkar University, Agra Uttar
Pradesh, India

Darshika Nigam

Department of Biochemistry,
School of Life Sciences, Dr. B.R.
Ambedkar University, Agra
Uttar Pradesh, India

Comparative study on antioxidant activity, phytochemical analysis and mineral composition of the Mung Bean (*Vigna Radiata*) and its sprouts

Udita Tiwari, Ankit Servan and Darshika Nigam

Abstract

Mung beans have extensive applications in various industries such as agriculture, food, pharmaceutical and cosmetics industries. Mung bean seeds and sprouts are consumed as excellent functional food worldwide that lower the risk of numerous diseases. Keeping this in view, the objective of the present study is identification of secondary metabolites and determination of antioxidative activity, protein content and metal estimation in dry mung seed (Gr-1) and 3 days sprouted raw (Gr-2) and sprouted boiled (Gr-3) mung beans. Phytochemical analysis of all the three groups showed the presence of alkaloids, flavonoids, protein and amino acid, phytosterols, phenols and carbohydrate. Glycosides were only present in dry mung seed. Total phenolic contents in 10% aqueous homogenates of all three mung samples were found 10.5 ± 0.91 mg, 39 ± 1.28 mg & 8.6 ± 0.26 mg in Gr 1, Gr 2 & Gr 3, respectively. Antioxidant activity measured as ferric reducing antioxidant power assay showed $7.6 \pm 0.35\%$ inhibition in Gr 1, $4.4 \pm 0.98\%$ inhibition in Gr 2 and $2 \pm 0.11\%$ inhibition in Gr 3. Total protein estimation was conducted by Lowry's method showed $100 \text{g} \pm 8.5$, $500 \text{g} \pm 40.5$ and $50 \text{g} \pm 3.6$ protein per 100 g of sample in Gr1, Gr 2, Gr 3, respectively. Since trace elements play both curative and preventive role in combating diseases, therefore, further metal estimation (P,K,Na,Zn,Cu,Fe,Mg and Ca) was conducted in dry mung seed and 3 days sprouted mung bean samples. Ca was found in highest concentration (601.00 ± 30.01 ppm) dry mung seed sample, while P element was found in lowest concentration in both the samples. Fe concentration (759.00 ± 15.91 ppm) was found highest in sprouted mung sample.

Therefore, it may be concluded that presence of high contents of protein, antioxidant activity, phenolic compounds and microelements, raw mung sprouts can be recommended for functional ingredients, as well as an excellent dietary source of antioxidants. However, boiling of sprouted beans reduces nutritional value.

Keywords: *Vigna radiata*, antioxidant, phytochemical

1. Introduction

Natural compounds, especially derived from dietary sources provide a large number of antioxidants, which act as radical scavengers and help in converting the radicals to less reactive species. The substances which neutralize the free radicals or their actions are known as antioxidants^[1]. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables, tea etc.^[2]. Reactive oxygen species and reactive nitrogen species (RNS), such as hydroxyl radical ($\bullet\text{OH}$), hydrogen peroxide (H_2O_2), superoxide ($\text{O}_2\bullet^-$), nitric oxide ($\text{NO}\bullet$), peroxyxynitrite (ONOO^-), and others, are major sources of oxidative stress in cells, damaging proteins, lipids, and DNA^[3]. This oxidative damage is considered to play a causative role in aging and several degenerative diseases associated with it, such as heart disease, cataracts, cognitive dysfunction, and cancer. Mung bean (*Vigna radiata*), also called green gram is a tropical legume. Mung bean use has long been regarded in the oriental dishes as a healthy food which increases energy and prevents aging.

Mung contains plenteous biological activities to prevent human diseases^[1]. Mung bean becomes more enriched with metabolites and activities after germination. The beans are rich source of protein and amino acid especially lysine and thus is used as functional food supplement along with cereal-based human diets. It is also a good source of thiamin, niacin, vitamin B6, pantothenic acid, magnesium, iron, phosphorus and potassium, and a very good source of dietary fiber, vitamin C, riboflavin, folate, vitamin K, copper and manganese. It is low in saturated fat and sodium, and very low in cholesterol. Soluble fiber can help lower blood cholesterol^[4]. High levels of amino acids, proteins, polyphenols and oligosaccharides in mung beans are thought to be the main contributors of the antioxidative, anti-inflammatory, antimicrobial and antitumor activities of this food and play role in the regulation of lipid metabolism^[5].

Correspondence**Darshika Nigam**

Department of Biochemistry,
School of Life Sciences, Dr. B.R.
Ambedkar University, Agra
Uttar Pradesh, India

The aim of the present study was to investigate the comparative analyses of dry, raw sprouted and boiled sprouted mung bean for antioxidant activity, total phenolic compound contents and protein contents as health promoting factors.

Materials and Methods

Collection of mung seeds

Mung seed (*Vigna radiata*) were procured from the National Seed Corporation, Agra (U.P.).

Chemicals

All the chemicals used in this study were of analytical grade. Folin–ciocalteu, gallic acid, potassium ferricyanide, sodium carbonate, ferric chloride, methanol (HPLC grade), etc. were purchased from Merck (Germany) and Sigma-Aldrich. (St. Louis, Mo, USA). Antibiotics and reagents for culture media were procured from Hi Media, Mumbai, India.

Instruments

Important equipments used in the present study were spectrophotometer (Electronics Corporation of India Limited), centrifuge (Remi, India), autoclave, hot air oven (Scientific equipment works), electronic analytical balance (Sartorius, Germany), laminar flow (Swastik India), incubator (Toshiba), deep freezer, micropipettes (Eppendorf, Germany), pH meter, etc.

Methods

Preparation of mung samples

Mung seeds were first washed with tap water thoroughly and finally with deionized water. Seeds were dried in air. Following groups were made to carry out experiments:

Sample preparation for biochemical analyses

Group 1: Dried mung seeds were crushed in an electric grinder to make fine powder.

Group 2: Mung seeds were soaked in distilled water over night. On next day soaked seeds were tied in muslin cloth for 3 days. Cloth containing seeds was kept moist by spraying distilled water at intervals of 6 hours every day to germinate mung seeds. Sprouted raw mung seeds were used for further biochemical analyses.

Group 3: Sprouted seeds (same as group 2) were boiled for further biochemical analyses.

10 % homogenate was prepared in distilled water for all the three samples for various biochemical analyses.

Sample preparation for metal analyses

Group 1: Dried mung seeds were crushed in an electric grinder to make fine powder.

Group 2: Sprouted mung seeds were prepared same as discussed above for group 2 (except seeds were soaked in deionized water). On 4th days sprouted seeds were kept in oven at 60°C in paper bags until the seeds were completely dried.

Samples of both groups were digested for metal analyses as discussed in method.

Phytochemical screening

Various phytochemicals such as phytosterol, tannins, phenols, flavonoids, alkaloids, glycosides, saponins, carbohydrates and proteins were detected in the extracts according to the procedures followed by [6].

Determination of total phenolic content (TPC)

The total phenolic content of seeds extract was determined spectrometrically as described by Singleton and Rossi [7] with little modification. According to this, 1ml of distilled water seed extract was mixed with 5ml of folin-ciocalteu reagent (diluted to tenfolds) and 4 ml of Na₂CO₃ (75g/l) and 10 ml of distilled water. The mixture was allowed to stand at 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 minutes and the absorbance of supernatant was taken at 760 nm using UV-VIS spectrometer. Different concentration ranges from 20, 30, 40, 60, 80, 100 µg/ml methanolic gallic acid solution were used as standard. Results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight of sample. All determinations were performed in triplicate.

Ferric reducing antioxidant assay power (FRAP)

The reducing power of aqueous mung seed extract was determined by the method of [8]. According to this, 1ml of seeds extract was mixed with 1 ml of distilled water and then added phosphate buffer (2.5ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. Then 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and FeCl₃ (0.5ml, 0.1%) and then absorbance was measured at 700nm against a blank using UV-Vis spectrophotometer and compared with ascorbic acid as standard. Results were expressed as percent inhibition which was calculated using the following expression:

% inhibition = $(A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$, where A_{blank} and A_{sample} stand for absorption of blank sample and absorption of tested extract solution, respectively. All determinations were performed in triplicate.

Total protein content

Protein contents were estimated in 10% homogenate (w/v) of raw moong seed (Gr.1), raw 3 d sprouted mung (Gr.2) and boiled 3 d sprouted mung (Gr.3) by the method of Lowry [9] using 0.2 mg/ml BSA as a standard. TCA precipitated proteins were dissolved in 1N NaOH and 0.2 ml of protein aliquot was pre-incubated with 0.8 ml distilled water and 5 ml alkaline copper reagent for 10 minutes at room temperature. Finally, 0.5 ml 1N Folin reagent was added and kept again for 30 minutes at room temperature. The optical density (O.D) of solution was read at 750 nm and protein contents were expressed as mg protein/g of mung seed.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for three parallel measurements using Graph Pad Prism version 7.02 for windows, Graph Pad Software, San Diego, California, USA. Statistical analysis was done by one way ANOVA and $p < 0.05$ considered as significant.

Results and Discussion

Phytochemical screening

In the present investigation, preliminary phytochemical analysis of all the three groups showed the presence of alkaloids, flavonoids, protein and amino acids, phytosterols, carbohydrate. Glycosides are only present in dry mung seed, while tannins and phenols were absent in all the three groups (shown in table 1). Plant based phenolic compounds exhibit antioxidant activity by scavenging the free radicals generated

during the normal metabolic process. This group encompasses a wide diversity of compounds, which mainly includes flavonoids and proanthocyanidins (condensed tannins) the antimicrobial activity due to flavonoids may be because of their structure, as they have the ability to form a combined

complex with bacterial cell walls. Increased antimicrobial activity reported that the sites and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms [10].

Table 1: Table showing phytochemicals present in various mung samples

Phytochemical	Name of phytochemical test	Dry mung seeds (Gr-1)	Raw sprouted mung seeds (Gr-2)	Boiled sprouted mung seeds (Gr-3)
Alkaloids	Mayers & Dragendorff's	+	+	+
Glycosides	Keller Kilani	+	-	-
Flavonoids	Shinoda's & Zn-HCl	+	+	+
Proteins & Amino acids	Ninhydrin, Biuret	++	+++	+
Phytosterols	Salkowski & Liebermann Burchad	+	+	+
Carbohydrates	Benedicts and Fehling	+	+	+
Tannin & phenol	Ferric chloride,	-	-	-

Total phenolic content and reducing antioxidant power

Free radicals alter many crucial biomolecules resulting into several types of diseases including cardiovascular diseases, neurodegenerative diseases, aging, cancer, etc. Antioxidants act against free radicals and protect the body [11]. Natural compounds especially polyphenolics are widely considered to carry antioxidant components and thought to cure the damage free radicals [12, 13]. In such consideration total, free and bound phenolics were extracted from raw and sprouted mung beans. The phenolics can be withdrawn from the dried samples depending on the solvent and extraction type. The total phenolics content of raw and sprouted mung beans were evaluated as gallic acid equivalents by Folin-Ciocalteu method. This assay measures the ability of phenolics to transfer electrons in the alkaline environment by subsequent formation of phosphor molybic or phosphor tungstic complexes.

Fig 1 shows, total phenolic contents in 10% aqueous homogenates of all three mung samples were measured as mg equivalents of gallic acid per gm of sample which were found 10.5 ± 0.91 mg, 39 ± 1.28 mg & 8.6 ± 0.26 mg in Gr 1, Gr 2 & Gr 3, respectively (as shown in figure 1).

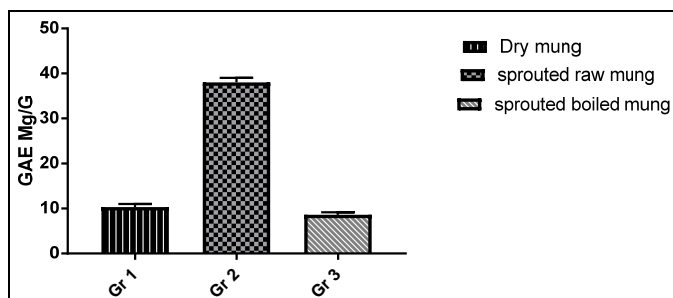


Fig 1: Graph showing total phenolic acid content in Gr.1 (dry mung seed), Gr.2 (sprouted raw mung) and Gr.3 (sprouted boiled mung)

Our results were in accordance to finding of other researchers that reported sprouted mung beans contain higher phenolics than raw seeds [14-16]. Sprouting naturally enhance the nutritive value of legumes due to increase in phenolic contents. It may be possible due to enzymatic degradation of carbohydrates resulting into polyphenols production. Phenolic compounds are a major group of compounds that contributed to the antioxidant activity and probably the mechanism of radical scavenging is responsible for it [17, 18].

Phenolic compounds have been reported to reduce the risk of cancer, heart disease, and diabetes, as well as have antibacterial, antiviral, anti-inflammatory, and anti-allergenic

activities [19-21].

Phenolic acids have complex structures as these compounds may exist in multiple forms as free, esterified, glycosylated or polymerized makes it difficult to separate and quantify individual antioxidants [13, 22]. Yao *et al* [23] reported that mung beans contain both types of phenolics i.e. free and bound phenolics. Tang *et al* [5] used shikimic acid and caffeic acid as chemical markers and a good linearity was discovered suggesting that germination of mung beans probably increase the levels of these two phenolics compounds.

Ferric reducing antioxidant power

The reducing power of the complex, which may serve as a significant reflection of antioxidant activity, was determined using a modified Fe (III) to Fe (II) reduction assay. The presence of antioxidants in the samples causes the reduction of the Fe³⁺/Ferricyanide complex to the ferrous form. Therefore, Fe²⁺ can be monitored by measuring of the formation of Perl's Prussian blue at 700 nm [24].

In the present investigation, figure 2 is the graphical representation of antioxidant activity measured as ferric reducing antioxidant power assay showed 7.6% ±0.35 inhibition in Gr 1, 4.4%±0.98 inhibition in Gr 2 and 2% ±0.11inhibition in Gr 3, while percent inhibition for ascorbic acid which was used as standard 10% ±2.67. Present results of reducing power indicates that boiling of sprouted mung bean decreases the antioxidant activity which may be due to denaturation of antioxidative proteins and enzymes. Many researchers have also shown that high temperature during germination decreases many vitamins, secondary metabolites and protein contents in soya beans and food grains [25, 26].

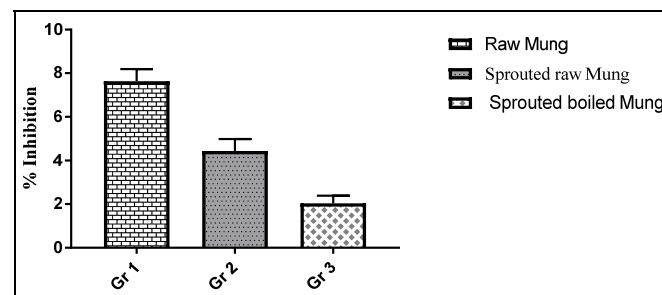


Fig 2: Graph showing reducing power in Gr.1 (dry mung seed), Gr.2 (sprouted raw mung) and Gr.3 (sprouted boiled mung)

Total protein contents

In the present study, total protein was done by Lowry's method showed 100g ±8.5 in Gr 1 sample, in Gr 2 500g±40.5

and $50g \pm 3.6$ protein in Gr 3 sample. In Gr 3, high temperature significantly decreases the total protein in comparison to Gr2 sample.

Mung bean protein is rich in essential amino acids, such as total aromatic amino acids, leucine, isoleucine and valine, while slightly deficient in threonine, total sulfur amino acids, lysine, and tryptophan as compared with the reference pattern. However, during sprouting, the proteolytic cleavage of proteins leads to a significant rise in the levels of amino acids [5].

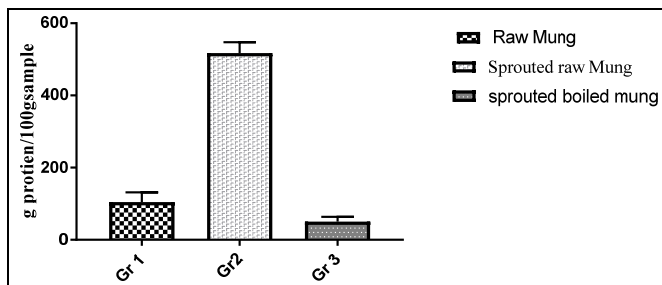


Fig 3: Graph showing total protein content in Gr.1 (dry mung seed), Gr.2 (raw sprouted mung) and Gr.3 (boiled sprouted mung)

Mineral composition

The concentrations of minerals (in mg/g) in dry mung seed, sprouted mung seed were determined by AAS. In the present study, the analysis for various elements indicated that K, Ca, Mn, Fe, Cu, Na, P and Zn were present in both samples of mung beans which are responsible for curing many diseases as shown in Table 1. These elements play a vital role in the formation of secondary metabolites which are responsible for pharmacological actions of medicinal plants.

Trace elements play both curative and preventive role in combating diseases. There is a vast scope to exploit the preventive medicinal aspects of various trace elements such as Cu, Cr etc. [27]. The level of iron (Fe) as shown in Table 3 with values 118.40 ± 6.77 and 759.00 ± 15.91 ppm in dry mung

seed and sprouted mung, respectively. It is an essential mineral to prevent anemia and cough associated with angiotensin-converting enzyme (ACE) inhibitors [28]. As shown in Table 3, Ca is the most abundant element in dry mung (601 ± 30.01 ppm) and in sprouted mung seed (594 ± 28.44 ppm). Calcium overcome the problems of high blood pressure, heart attack, premenstrual syndrome, colon cancer and keeping the bones strong and reduces the risks of osteoporosis in old age [29]. Manganese (Mn) was found at the concentration 11.20 ± 0.82 and 17.40 ± 0.95 ppm in dry mung seed and sprouted mung, respectively. Mn can help to assist the body in metabolizing protein, help the diabetic also metabolize carbohydrates and in treating diabetes [30]. Zn deficiency may contribute to arrested sexual maturation, growth retardation and hair loss, delayed wound healing and emotional disturbance [31]. In the present study, higher concentration of Zn in dry mung (60 ± 3.12 ppm) than sprouted mung seed (67 ± 3.20 ppm). K is helpful in reducing hypertension and maintaining cardiac rhythm. In the human body, the elements play vital role in many physiological reactions and their deficiency or excess can affect human health [32]. Present study showed almost same concentration of K is found in both the samples.

Sodium (Na) involves in the production of energy, transport of amino acids and glucose into the body cells [33]. The concentration of Na was found 26.2 ± 1.25 in dry mung seeds, while in 25.6 ± 1.31 was found in sprouted mung seeds. Copper (Cu) play important role in treatment of chest wounds and prevent inflammation in arthritis and similar diseases [34]. In present investigation, Cu concentration was higher in sprouted mung seed (9.2 ± 0.58) than dry mung seeds (6.3 ± 0.34). The concentration of phosphorus was found 0.3 ± 0.03 in dry mung seeds, while in 0.5 ± 0.031 was found in sprouted mung seeds. Phosphorus plays a major role in the structural framework of DNA and RNA. Living cells use phosphate to transport cellular energy with adenosine triphosphate (ATP), necessary for every cellular process that uses energy [35].

Table 1: Average concentration of different metals in the sample analyzed

Sample	Phosphorus (ppm)	Potassium (ppm)	Sodium (ppm)	Zinc (ppm)	Copper (ppm)	Iron (ppm)	Manganese (ppm)	Calcium (ppm)
Dry Mung	0.3 ± 0.03	0.72 ± 0.05	26.20 ± 1.25	60.00 ± 3.12	6.30 ± 0.34	118.40 ± 6.77	11.20 ± 0.82	601.00 ± 30.01
Sprouted Mung	0.5 ± 0.031	0.7 ± 0.04	25.60 ± 1.31	67.00 ± 3.20	9.20 ± 0.58	759.00 ± 15.91	17.40 ± 0.95	594.00 ± 28.44

Conclusion

Based on these results, it is concluded that presence of high contents of protein, antioxidant activity and phenolic compounds, make raw mung sprouts as functional food ingredients, as well as an excellent dietary source of antioxidants. Further studies may focus on the extraction and purification of new physiologically active substances in mung seeds which may be useful in pharmaceutical purposes.

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