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COMPARATIVE NUTRACEUTICAL ANALYSIS OF SPROUTS AND MICROGREENS OF *VIGNA RADIATA* (MOONG DAL), *CICER ARIETINUM* (CHICK PEA), *PHASEOLUS VULGARIS* (RAJMA), AND *VIGNA MUNGO* (URAD DAL)

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ABSTRACT

Worldwide, there has been increasing shift in healthy eating, especially fresh, ready-to-eat, functional food, such as sprouted seeds and microgreens. The present study describes the comparative analysis of carbohydrates, proteins, phenolics, flavonoids and antioxidants present in sprouts and micro-greens of *Vigna radiata* (moong dal)*, Cicer arietinum* (chick pea), *Phaseolus vulgaris* (rajma)*, and Vigna mungo* (urad dal). The content of phytochemicals presents in the legumes change during different growth stages of plant growth and development. In the present study we found that sprouts of *Phaseolus vulgaris* (rajma) had the highest amount of antioxidants (1.7357mgAAE/gm), but *Vigna mungo* (urad dal) sprouts were found to contain the highest amount of phenolics (10.432mgTAE/gm). In case of microgreens, the highest protein levels were found in *Cicer arietinum* (chick pea) microgreens (2.315mg/ml). The sprouts of *Vigna mungo* (urad dal) were found to be containing the highest amount of ascorbic acid (0.9mg/ml). It was found that *Vigna mungo* (urad dal) contained the highest amount of chlorophyll during its sprouting stage (30.279mg/g). The microgreens of *Cicer arietinum* (chick pea) and *Phaseolus vulgaris* (rajma) were found to contain similar

amounts of reducing sugars (215.82mg/ml) which were higher than the reducing sugars present in micro-greens and sprouts of urad and moong dal. The sprouts of *Vigna mungo* (urad dal) were found to contain the highest amount of flavonoids (97.08mgAAE/gm).

Keywords: antioxidants, nutraceutical, sprouts, microgreens, *Vigna radiata* (moong dal), *Cicer arietinum* (chick pea), *Phaseolus vulgaris* (rajma), *Vigna mungo* (urad dal)

INTRODUCTION

According to WHO, majority of the nations in the world are being affected by triple burden of malnutrition i.e., under-nutrition, nutrient deficiency and over-nutrition. Due to urbanization, production of processed foods is increasing which is leading to a shift towards unhealthier dietary patterns. These days, foods with higher carbohydrates, fats, free sugars, and salts are being consumed by people around the globe. Overweight and obesity have emerged as severe public health problems leading to non-communicable diseases (NCD). In 2017, about 63% of deaths in India were attributable to NCDs **[1]**.

However, recently awareness about healthy diets has led to increased consumption of microgreens and sprouts which can be considered as fresh and ready-to-eat functional foods **[2]**. They have various phyto-chemicals which enhance human diet and eradicate nutrient deficiencies **[3]**. The quantity of anti-nutritive compounds e.g., trypsin inhibitor, phytic acid, pentosan, tannin, and cyanides are decreased, whereas bioavailability of phytochemicals such as glucosinolates and natural antioxidants are increased during the germination of the seed **[4]**.

Sprouting is defined as process of germination of seeds to give out shoots and roots when they are soaked in water for specific hours and right temperature and appropriate environment. The nutrition level is higher in sprouts as compared to the fully grown plants. Microgreens are vegetable greens that are just used after the cotyledon leaves have developed with one set of true leaves. They are being used as nutritional supplements as they provide flavour and enhance the texture of food and also add sweetness and spiciness to foods. Micro-greens embrace a high-level nutritional profile and exhibit several health benefits. The content of phytochemicals like phenolic compounds and carotenoids, vitamins like Vitamin C and minerals like zinc and copper is high in micro-greens.

Pulses form an essential component of the daily diet of Indians who are predominantly vegetarians. It provides all the essential amino acids in requisite amounts and helps eliminate protein-calorie malnutrition.

The germination increases the phytochemical content of the legumes. The aim of this work was to characterize and compare natural antioxidants such as L-ascorbic acid, phenolic, flavonoid and anti-antioxidant compounds in sprouts and microgreens of common Indian pulses such as *Vigna radiata* (moong dal), *Cicer arietinum* (chick pea), *Vigna mungo* (urad dal) and *Phaseolus vulgaris (*rajma). The present also analysed the nutritional values of sprouts and microgreens in terms protein, sugar, and chlorophyll content.

MATERIAL AND METHODS

2.1 Plant material

Seeds of *Vigna radiata* (moong dal)*, Cicer arietinum* (chick pea)*, Vigna mungo* (urad dal) *and Phaseolus vulgaris* (rajma) were collected from a local mill. All these seeds were sterilized using 2% of Bavistin to avoid fungal infection. The soaked grains seeds were sown in triplicates in pots having normal soil. The Watering of pots was done regularly to keep the soil moist. The sprouting and germination of the seeds were carried out in an uncontrolled temperature (average 24-25°C) with daylight. The room was subjected to a diurnal cycle with fluctuations of natural temperature, humidity, and light.

2.2 Preparation of sample

The sprouts and microgreens were harvested on day 5^{th} and 10^{th} day respectively after sowing and all the suspended dirt particles were thoroughly removed. Nearly 1-2gm of sample was weighed and washed. The leaves were crushed using a mortar/ pestle and dissolved properly in 10ml of sterilized water to make the mixture.

2.3 Estimation of total protein content

The total protein content was determined spectrophotometrically at 750 nm using Bovine Serum Albumin (BSA) as a standard **[5]**. The sample extract (1ml) was added to test tubes containing 1ml of distilled water and 5ml of alkaline solution.0.5 ml of Folin-Ciocalteau solution was added to each test tube and incubated for 30 minutes at room temperature. The total protein content was calculated & results were expressed in mg/ml.

2.4 Estimation of total phenol content (TPC)

The total phenol content (TPC) was determined spectrophotometrically at wavelength 760nm using tannic acid as a standard **[6]**. To the tubes containing 0.125ml of Folin-Ciocalteu's reagent and 2.5ml of distilled water, 0.2ml of the diluted sample extract (in triplicate). Then, 1.25ml of 7% sodium carbonate solution was added and incubated at room temperature for 90 minutes. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of tannic acid equivalent per g dry weight (mg TAE/g).

2.5 Determination of Total flavonoid content

Total flavonoid content was measured by the modified aluminium chloride colorimetric assay **[6]**. The reaction mixture consisted of 1.0ml of extract and 4ml of distilled water taken in the test tube. To it 0.30ml of 5% sodium nitrite was added and after 5 minutes, 0.3 ml of 10% aluminium chloride was mixed. After 5 minutes, 2.0ml of 1M Sodium hydroxide was added and double-distilled water was added to make final volume to 10ml. The absorbance for test and standard solutions were determined against the reagent blank at wavelength 510nm with an UV/Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve and was expressed as mg Ascorbic acid equivalent (AAE)/g of extract.

2.6 Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The modified FRAP assay, was used to evaluate the total antioxidant capacity spectrophotometry **[6]**. 0.9 ml of ethanol, 5.0 ml of distilled water, 1.5 ml of 1M HCl, 1.5 ml of 1% potassium ferricyanide, 0.5 ml of 1% SDS, and 0.5 ml of 0.2% ferric chloride were added to 0.1 ml of extract. The mixture was boiled in a water bath at 50°C for 20 minutes and cooled rapidly. The absorbance was measured at wavelength 750nm to measure the reducing power of the extract The ascorbic acid standard curve was used to calculate the antioxidant content of the samples, and the results were represented as mg ascorbic acid equivalent (mg AAE)/g.

2.7 Estimation of ascorbic acid

The Ascorbic acid was measured spectrophotometrically by 2,4-DNPH method at wavelength 540nm and expressed in mg/ml **[6]**. To all the test tubes containing 0.3ml of extract, distilled water was added to make up to 1.5ml. 0.5 ml of 2, 4- DNPH was added and after proper mixing, test tubes were incubated at 37º C for 3 hours. 3.5ml of 80% H_2SO_4 was added to the test tubes to dissolve the orange red osazone crystals formed.

2.8 Estimation of Reducing sugars

Sugar acts as a reducing agent that reduces 3,5-dinitrosalicylic acid under alkaline medium to form an orange red coloured product which was measured spectrophotometrically at 540nm.1ml of extract was taken in test tube **[7]**. The distilled water was added so as to make the volume up to 3ml. 1ml of DNS reagent was added in each test tube and incubate it for 15 min in boiling water bath. The test tubes were cooled & absorbance was measured.

2.10 Statistical Analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one-way ANOVA ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

The microgreens of *Phaseolus vulgaris* (rajma) (215.82 mg/ml), *Cicer arietinum* (chick pea) (215.82 mg/ml), *Vigna radiata* (moong dal) (204.46 mg/ml) and *Vigna mungo* (urad dal) (209.78 mg/ml) had higher values of reducing sugars such as glucose and maltose as compared to their sprouts (6.76 mg/ml, 4.014 mg/ml, 7.726 mg/ml, 10.848 mg/ml respectively). These sugars act as growth-related functions and also serve as signaling molecules that modulate light signaling pathways. Thus, it can be implied that microgreens have more digestibility owing to the presence of soluble sugars.

The protein levels were higher in microgreens of *Phaseolus vulgaris* (rajma) (2.083mg/ml) and *Cicer arietinum* (chick pea) (2.315mg/ml) as compared to their sprouts (1.35mg/ml, 1.079mg/ml respectively) whereas in *Vigna radiata* (moong dal*)* the protein levels were similar in sprouts (1.813mg/ml) and microgreens (1.743mg/ml). The *Vigna mungo* (urad dal) sprouts (2.083mg/ml) had higher protein levels in comparison to its microgreens (1.550mg/ml) ($p \leq 0.05$) as reported earlier **[8]**. The level of proteins was found to be significantly higher in *Phaseolus vulgaris* (rajma) microgreens as compared to sprouts ($p \leq 0.05$). Various amino acids are present in sprouts and microgreens for example L-asparagine, L-ornithine, L-glutamine, L-proline, L-methionine, L-cysteine. The lentils such as *Vigna radiata* (moong dal)*, Cicer arietinum* (chick pea)*, Vigna mungo* (urad dal) *and Phaseolus vulgaris* (rajma) are rich in L-asparagine and L-ornithine. The amino acids present in the sprouts and microgreens are produced by the hydrolysis of protein reserves which are used for the synthesis of new components. The significant variations in the concentrations of amino acids in sprouts and microgreens could be due to the metabolic changes during growth and ripening. The observed decreased levels of protein in microgreens could be due to the mobilization of protein reserves in cotyledons and their utilization in synthesis of new proteins needed for growth of sprouts to microgreens **[9]**.

The chlorophyll content in sprouts ranged from 30.279 mg/g to 3.237 mg/g and decreased in the order: *Vigna mungo>Vigna radiata >Phaseolus vulgaris >Cicer arietinum*. The chlorophyll content in microgreens range from 7.512 mg/g to 3.237 mg/g. It decreased in order as *Cicer arietinum > Phaseolus vulgaris > Vigna radiata >Vigna mungo.* The chlorophyll content in *Phaseolus vulgaris* (rajma) sprouts (9.44 mg/g) was found to be higher than its microgreens (5.843 mg/g). However, in *Cicer arietinum* (chick pea) microgreens (7.512 mg/g) the chlorophyll content was higher than sprouts (3.44 mg/g). The *Vigna radiata* (moong dal) sprouts (16.422 mg/g) have higher chlorophyll content than microgreens (3.443 mg/g). *Vigna mungo* (urad dal) sprouts (30.279 mg/g) had higher chlorophyll content than microgreens (3.237mg/g). Since the sprouts and microgreens were drawn in controlled environmental growth conditions, the increased levels of chlorophyll in sprouts are beneficial for plant growth and development by enhancing cellular metabolism and synthesis of defense related secondary metabolites. The higher level of chlorophyll in microgreens of Cicer arietinum (chick pea) is in accordance with the various other studies who reported increased chlorophyll content in amaranth, green peas, beet root, radish and kale **[8]**.

The levels of ascorbic acid were significantly higher in sprouts (0.79mg/ml, 0.9mg/ml, 0.69mg/ml, 0.7mg/ml respectively) of *Phaseolus vulgaris*, *Vigna mungo*, *Vigna radiata*, *Cicer arietinum* as compared to their microgreens (0.3mg/ml, 0.24mg/ml, 0.12mg/ml, 0.17 mg/ml respectively) ($p \leq 0.05$). Similar results were observed in another study who found higher ascorbic levels in sprouts of broccoli and radish as compared to microgreens **[8]**. It has been found that the ascorbic acid levels increase during germination due to intense synthesis of L-ascorbic acid and then this level is maintained at a constant level after sprouting. Thus, sprouts can be a good source of vitamin C as compared to microgreens.

The phenolic levels in sprouts ranged from 10.432mgTAE/gm to 5.277mgTAE/gm. It decreased in the order *Vigna mungo>Phaseolus vulgaris >Vigna radiata >Cicer arietinum.* The phenolic levels of microgreens ranged from 3.8mgTAE/ml to 3.051mgTAE/gm. It decreased in the order as *Phaseolus vulgaris >Cicer arietinum >Vigna radiata >Vigna mungo.* The phenolics were higher in sprouts of *Phaseolus vulgaris (*6.896mgTAE/gm), *Cicer arietinum (*5.277mgTAE/gm), *Vigna radiata* (6.579mgTAE/gm) and *Vigna mungo* (10.432mgTAE/gm) as compared to microgreens of *Phaseolus vulgaris (*3.8mgTAE/gm*), Cicer arietinum (*3.226mgTAE/gm*), Vigna radiata (*3.094mgTAE/gm*), Vigna mungo (*3.051mgTAE/gm*)* respectively. *Phaseolus vulgaris* (rajma)*, Cicer arietinum* (chick pea)*, Vigna radiata* (moong dal) sprouts had significantly higher values of phenolics than microgreens ($p \leq 0.05$). Similarly, in another study, it was observed that phenolic levels were higher in sprouts of radish, lentil, broccoli and sunflower as compared to their microgreens **[8]**. The phenolics present in *Vigna radiata* (moong dal) are p-Coumaric acid, Caffeic acid, t-ferulic acid and in *Phaseolus vulgaris* (rajma)*,* phenolics present are hydroxyl-benzoic acid such as gallic acid, p-Coumaric acid, sinapic acid, Caffeic acid, pelargonidin and cinnamic acid. The phenolics present in *Cicer arietinum* (chick pea) are Gomisin D and Anhydro-secoisolariciresinol, Pelargonidin 3,5-*O*-diglucoside, Hesperetin 3′,7-*O*-diglucuronide, 6-Geranylnaringenin, Isorhamnetin, Phloretin 2′-*O*-glucoside, *p*-Coumaroyl glucose, 3,4-Diferuloylquinic acid, Hydroxytyrosol 4-*O*-glucoside and 1,4-Naphtoquinon. The low levels of phenolics observed in microgreens could be due to differences in harvest stage as compositional changes are known to take place during the seed-sprout-microgreens-mature plant **[10]**. It has been found that there is significant phenolic transformation during plant ontogenesis. During the synthesis of phenolic compounds L-phenylalanine is converted into trans-cinnamic acid by phenylalanine ammonia lyase leading to the synthesis of phenolic acid and flavan-3-ols present in sprouts and microgreens.

The level of flavonoids was higher in *Phaseolus vulgaris* (rajma) micro greens (6.66mgAAE/gm) than sprouts (5.42mgAAE/gm), whereas in *Cicer arietinum* (chick pea), *Vigna mungo* (urad dal) and *Vigna radiata* (moong dal) flavonoids were higher in sprouts (7.02mgAAE/gm, 7.08mgAAE/gm, 5.61mgAAE/gm respectively) in comparison to their micro greens (5.15mgAAE/gm, 2.04mgAAE/gm, 2.40 mgAAE/gm respectively). The flavonoids were significantly higher in *Phaseolus vulgaris* (rajma) micro greens than its sprouts($p \leq 0.05$). Various flavonoids in *Phaseolus vulgaris* (rajma) are formononetin, genistein, quercetin, 3,4-diglucoside, spiraeside, and hyperoside **[11]**. The flavonoids present in *Vigna radiata* (moong dal) and *Vigna mungo* (urad dal) are vitexin, isovitexin, isovitexin-6″-*O*-α-L-glucoside, luteolin, quercetin, myricetin, Peonidin-3-glucoside, cyaniding-3-glucoside, pelargonidin-3-glucoside, luteolin, dulcinoside and kaempferol **[12]**. The flavonoids present in *Cicer arietinum* (chick pea) are formononetin glycoside, isoformononetin glycoside, biochanin-A, biochanin-A glycoside, biochanin-A malonyl glycoside, formononetin malonyl glycoside, Pelargonidin 3,5-*O*-diglucoside, Hesperetin 3′,7-*O*-diglucuronide, 6-Geranylnaringenin, Isorhamnetin and Phloretin 2′-*O*-glucoside **[13]**.

Various natural oxidants such as phenolics, ascorbic acid, flavonoids determine the antioxidant capacity of sprouts and microgreens. The antioxidants were found to be present in sprouts are in the range of 1.7357mgAAE/gm to 1.3607mgAAE/gm. The levels of antioxidants in sprouts decreased in order as *Phaseolus vulgaris* > *Vigna mungo*> *Vigna* *radiata* >*Cicer arietinum*. The range of antioxidants in microgreens was found to be from 1.675mgAAE/gm to 1.3821mgAAE/gm. The levels of antioxidants decreased in order as *Vigna mungo*>*Phaseolus vulgaris* > *Cicer arietinum* >*Vigna radiata.* The levels of antioxidants were higher in sprouts of *Phaseolus vulgaris* (1.7357mgAAE/gm) *and Vigna radiata* (1.5285mgAAE/gm) as compared to their) microgreens (1.4607mgAAE/gm, 1.3821mgAAE/gm respectively). The antioxidant levels in *Cicer arietinum* (chick pea) microgreens (1.4428mgAAE/gm) and *Vigna mungo* (urad dal) microgreens (1.675mgAAE/gm were higher than their sprouts (1.3607mgAAE/gm, 1.5821 mgAAE/gm respectively). The increased antioxidant levels in microgreen could be due to the presence of carotenoids, tocopherol and phenolic compounds such as glycosides of quercetin, luteolin and apigenin, hydroxyl benzoic acid and hydroxyl cinnamic acid present in legumes **[14]**. Thus, *Cicer arietinum* (chick pea) microgreens and *Vigna mungo* (urad dal) microgreens can be good natural source of antioxidants.

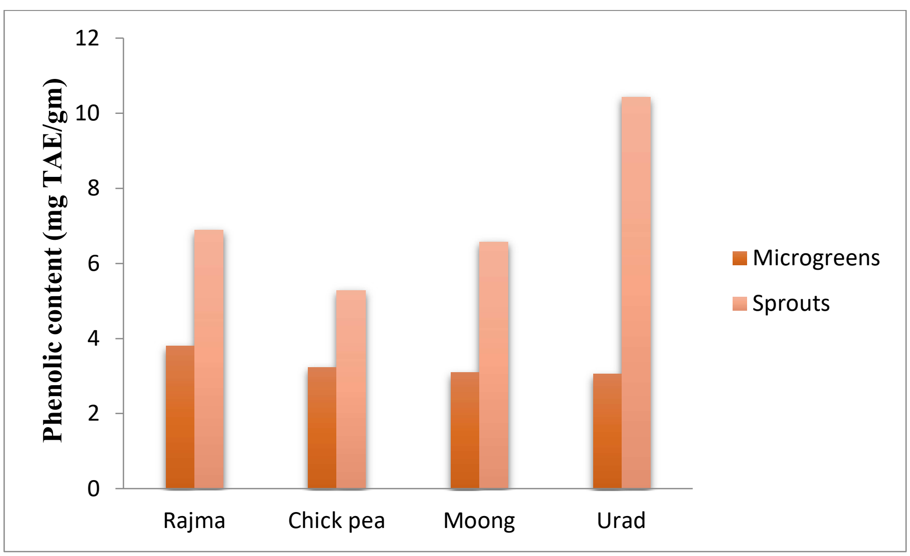

Figure 1: Graphical representation of comparative evaluation of phenolic content in microgreens and sprouts of *Phaseolus vulgaris* (rajma), *Cicer arietinum* (chick pea), *Vigna radiata* (moong dal) and *Vigna mungo (*urad dal*)*

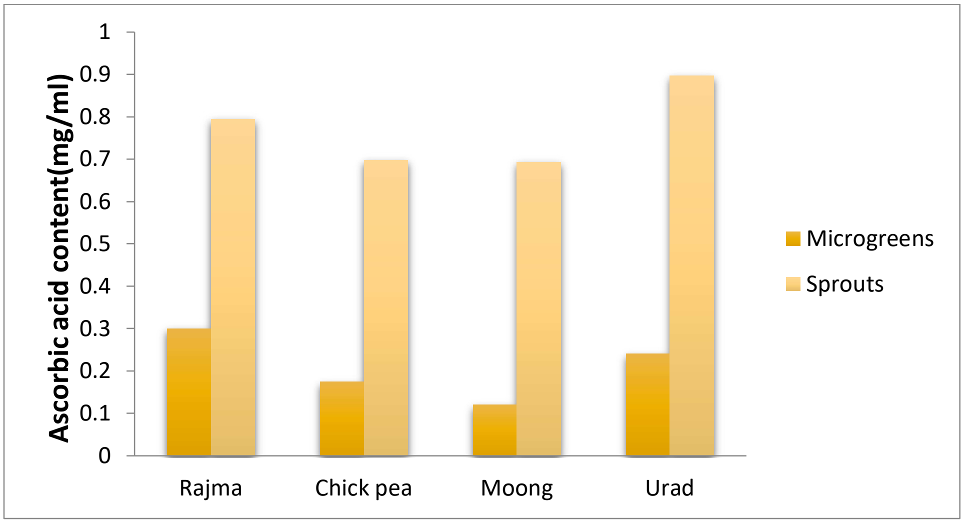

Figure 2: Graphical representation of comparative evaluation of ascorbic acid levels in microgreens and sprouts of *Phaseolus vulgaris* (rajma), *Cicer arietinum* (chick pea), *Vigna radiata* (moong dal) and *Vigna mungo* (urad dal)

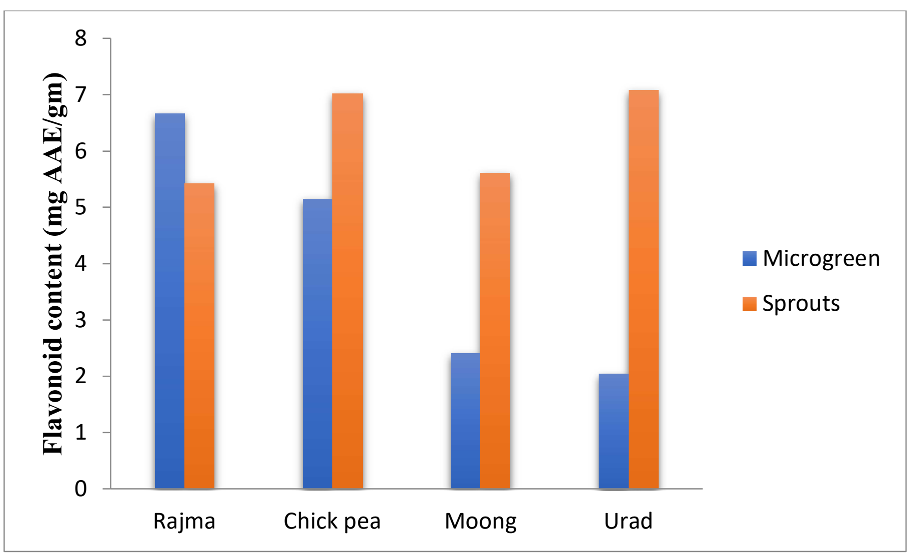

Figure 3: Graphical representation of comparative evaluation of flavonoid content in microgreens and sprouts of *Phaseolus vulgaris* (rajma), *Cicer arietinum* (chick pea), *Vigna radiata* (moong dal) and *Vigna mungo* (urad dal)

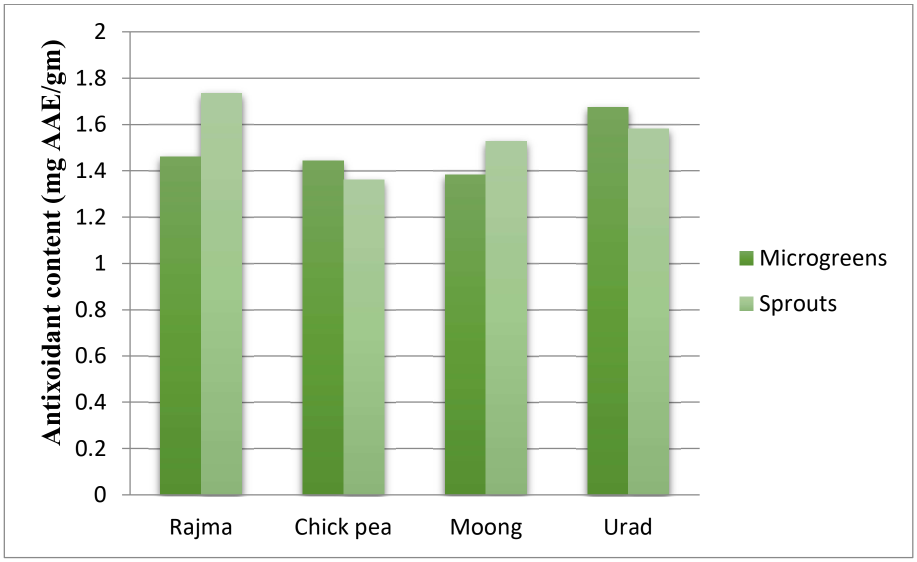

Figure 4: Graphical representation of comparative evaluation of antioxidant levels in microgreens and sprouts of *Phaseolus vulgaris* (rajma), *Cicer arietinum* (chick pea), *Vigna radiata* (moong dal) and *Vigna mungo* (urad dal)

CONCLUSION

Sprouts and microgreens are novel functional food sources with great potential as global food systems as they promote human health. In our study, we found that the microgreens of Rajma, Urad, Moong and Chickpea have more reducing sugars in comparison to their sprouts. In case of protein content, it was observed that rajma and chickpea microgreens had higher protein content whereas in case of moong, sprouts were observed to have higher protein content. The Chlorophyll content was found to be higher in the sprouts of Rajma, moong and urad in comparison to their microgreens, whereas in Chickpea microgreens, the protein content was higher than the chickpea sprouts. The ascorbic acid and phenolic levels were higher in sprouts of all the seeds as compared to their microgreens. Thus, sprouts can be a good source of vitamin C and phenols as compared to microgreens. The Flavonoids levels were higher in microgreens of Rajma as compared to their sprouts whereas in Chick pea, Urad and Moong, flavonoids were higher in sprouts when compared to their microgreens. It was found that the antioxidant levels were higher in sprouts of Rajma and moong as compared to their microgreens whereas, in chickpea and urad microgreens, the antioxidant levels were higher than their sprouts.

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