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RESEARCH ARTICLE

Nutraceutical profile of red gram genotypes with reference to phenols, phytic acid and antioxidant distribution

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ABSTRACT

Red gram is a widely consumed pulse and a good source of protein in the vegetarian diet. In addition to its nutritional qualities, it possesses therapeutic properties by virtue of the bioactive phytoconstituents, especially phenol. Phenols are a major group of secondary metabolites widely distributed in the plant kingdom. It occurs as derivatives or isomers of the various classes of phenols *viz.,* flavonoids, polyphenols, phenolic acids. The recent focus of phenols is the antioxidant potential that is responsible for several therapeutic effects of these compounds. The distribution of total phenols and flavonoids has been studied in seventy red gram lines, including short duration, mid duration, perennial and dual types. Total phenol content varied from 50 mg/100 g in ICPL 19003 and ICPL 19020 to 600 mg/100 g in the variety BSR-1. Total flavonoids range between 10 mg/100g to 80 mg/100g. Phytate content of the red gram genotypes ranged from 0.155 to 4.35mg/g on a dry weight basis and are indicated to be of therapeutic value. DPPH, ABTS, FRAP, and H_2O_2 radical scavenging activity of red gram genotypes were studied. Results indicate BSR-1, ICPL 19022, ICPL88039, and ICPL19036 have high antioxidant values. The nutraceutical properties expressed in red gram make it an ideal choice of pulse in therapeutic food in addition to its role as protein source.

Keywords:Red gram, total phenols, flavonoids, phytates, total antioxidants

INTRODUCTION

Red gram also called pigeon pea is the second important pulse crop next to chick pea and a main protein source in the vegetarian diet. Red gram seed is used as food and feed. Dual types are available in which the green pods are used as vegetables and the mature seed as pulse. Red gram is a good source of vegetable protein, in addition to minerals and vitamins. It also contains carbohydrates, especially oligosaccharides. Although red gram is consumed primarily as a source of protein, it also contains other phytochemicals that may be a potential source of bioactive principles. Anti nutritional factors in pulses include enzyme inhibitors, lectins, tannins, phytic acid, phenolics, oxalates, saponins and oligosaccharides, the distribution of which varies in different components of pulses. The cotyledons accumulate phytates, oligosaccharides and enzyme inhibitors, while the seed coat holds tannins and phenolic compounds (Dueñas *et al.,* 2002; Reddy *et al.,* 1985). Recognition of health benefits of some of these antinutritional factors has identified

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these as active ingredients in the food industry. This necessitates generation of scientific data on these antinutritional factors to go forward with use of pulses as functional food ingredients.

In recent years there is growing interest in developing functional foods with phenolic antioxidants. In this context, the present study was conducted to screen seventy red gram genotypes for total phenol content, flavonoid content, total antioxidant activity and free radical scavenging potential. In addition to this phytate content was assessed in the seventy red gram genotypes. This is of importance in the present day scenario where lifestyle diseases are prevalent and management of these conditions are easily achieved through the nutraceuticals present in the regular diet. In this context, screening for nutraceuticals in red gram seeds is significant as this is a widely consumed pulse and an important part of the Indian vegetarian diet.

MATERIALS AND METHODS

i) Collection of sample and extract preparation

Seventy red gram lines constituting twelve varieties including eleven COR and one BSR; thirty nine ICPL lines and nineteen genotypes including three IPAL, two WRGE, two PRG, two BRG and one line each of ICPWSMD 2201, PT12-5-5-1 , SKNP 1715, TS3R, GJP 2011, TDRG 272, Daftari Manik, BSMR, MAL 6, BWR23 *Cajanus cajan* L. were included in this study. Among these, three genotypes *viz*., PT12-5-5-1, SKNP 1715 and TS3R were short duration, one mid-early genotype GJP 2011, three genotypes *viz*., IPAL 21-1, IPAL21-17, IPAL 20-5 were medium duration, eight genotypes *viz*., ICPL 17103, WRGE 124, WRGE 134, TDRG 272, BRG 4, PRG 176, Daftari Manik, BSMR 316, were long duration. The samples also included two dual types *viz*., BRG 1, BWR23 suitable for vegetable purpose when tender and as seed on maturity. The varieties CO1R, 2R, 4R, 5R, 6R, 8R, 9R, 10R, 11R, 12R and 13R were sourced from NBPGR, New Delhi and BSR 1 from Agricultural Research Station, Bhavanisagar, TNAU. Seeds of thirty nine genotypes *viz*., ICPL 3, 7, 8, 9,12, 15, 17, 19, 20, 22, 23, 24, 25, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39, 41, 42, 43, 44, 46, 47, 48, 49, 87, 89, 151, 161, 88034, 88039, ICPWSMD 2201 were sourced from ICRISAT, Hyderabad. These seventy red gram lines were raised in the Department of Pulses, Millet Breeding Station, Centre for Plant Breeding and Genetics, Agriculture College and Research Institute, Tamil Nadu Agricultural University (TNAU), Coimbatore.

ii) Chemicals

Folin's reagent, sodium carbonate, methanol, aluminium chloride, DPPH, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, potassium persulfate, potassium ferriccyanide, trichloroacetic acid, ferric chloride, ferrozine, peroxidase, hydrogen peroxide were obtained from Sigma-Aldrich Germany. Sodium sulphate, sodium hydroxide, potassium thio cynate, were procured from Merck. All other chemicals were of Analytical grade.

iii) DPPH free radical scavenging activity

The free radical scavenging activity of each sample was evaluated using the method Jang *et al.,* 2017. 5 mL of a 0.1 mM DPPH solution in 95% ethanol was mixed with 1 mL of a tenfold diluted sample. The mixture was thoroughly shaken and incubated in the dark at room temperature for 30 minutes. After incubation, it was filtered through a 0.45 µm nylon syringe filter, and the absorbance was recorded at 517 nm. The DPPH radical scavenging activity was determined using the following formula.

Calculation:

% DPPH radical scavenging activity= $[(A_0-A_1)/_{A0}]$ X100

Where:

A_0 is the absorbance of the control

A_1 is the absorbance of the sample

iv) ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was determined using the method described by Re *et al*., 1999. Briefly, 2 mM ABTS was dissolved in distilled water containing 2.45 mM potassium persulfate and allowed to react in the dark at room temperature for 12 hours. The resulting ABTS solution was then adjusted with 0.1M sodium phosphate buffer (pH 7.4) to achieve an initial absorbance of approximately 0.75 ± 0.005 at 734 nm. Subsequently, 0.1 mL of each tenfold diluted sample was mixed with 3 mL of the ABTS solution. After incubating at room temperature for 10 minutes, the absorbance was measured at 734 nm. The ABTS radical scavenging activity was then calculated using the following equation.

Calculation:

% inhibition= $[(A_0-A_1)/_{A0}]$ X100

Where:

A_0 is the absorbance of the control

A_1 is the absorbance of the sample

v) FRAP total antioxidant activity

Total antioxidant activity was assessed using the FRAP assay, following a modified version of the Oyaizu method (1986). In this procedure, 1 mL of a tenfold diluted sample was combined with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 minutes. After incubation, 2.5 mL of 10% trichloroacetic acid was added, and the solution was centrifuged at 4000 rpm for 10 minutes. Next, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. After allowing the reaction to proceed at room temperature for 5 minutes, the absorbance was measured at 700 nm.

Vi) Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was assessed using a method Okamoto *et al.,* 1992. Briefly, 0.1 mL of each extract sample was mixed with 0.01 mL of 50 mM H_2O_2, 0.6 mL of peroxidase (10 U/mL), 0.6 mL of 0.1% ABTS, and 1.8 mL of 0.1 M phosphate buffer (pH 6.0). The mixture was then incubated at 37°C for 15 minutes. Following incubation, the absorbance of the solution was measured spectrophotometrically at 414 nm. A standard curve was generated using gallic acid equivalents.

vii) Estimation of total phenol

Total phenol content was determined using the Folin-Ciocalteu method, Singleton *et al.,* 1999. Briefly, 1.0 g of the sample was extracted in 10 mL of 80% ethanol and centrifuged at 10,000 rpm for 20 minutes to collect the supernatant. The extraction process was repeated, and the pooled supernatant was evaporated to dryness. The resulting residue was dissolved in 2 mL of distilled water, and 0.5 mL of the sample was taken for analysis. The volume was adjusted to 3.0 mL, followed by the addition of 0.5 mL of Folin-Ciocalteu reagent. After 3 minutes, 2 mL of 20% sodium carbonate was added, and the mixture was placed in a boiling water bath for one minute. The absorbance was then measured at 650 nm. A standard curve was prepared simultaneously using catechol solutions of varying concentrations.

viii) Estimation of total flavonoids

Total flavonoid content was determined using the method Jang *et al.,* 2017. In this procedure, 1.5 mL of 2% $AlCl_3$ was mixed with 0.5 mL of a tenfold diluted sample and allowed to react at room temperature for 10 minutes. The absorbance of the resulting mixture was then measured at 415 nm.

ix) Determination of phytic acid

Phytic acid was assessed using the method Abinisha & Ahuja, 2019. Weighed a finely ground (40-mesh) sample estimated to contain 30 mg of phytate phosphorus into a 125 mL Erlenmeyer flask. Extracted the sample using 50 mL of 3% TCA for 30 minutes with mechanical shaking for 45 minutes. Centrifuged the suspension and transferred a 10 mL aliquot of the supernatant to a 40 mL conical centrifuge tube. Quickly added 4 mL of $FeCl_3$ solution by rapidly expelling it from a pipette. Heated the mixture in a boiling water bath for 45 minutes. After 30 minutes, added 10 mL of 3% TCA and continued heating.

Centrifuged the tubes for 10 minutes and carefully decanted the clear supernatant. Washed the precipitate twice by thoroughly dispersing it in 20-25 mL of 3% TCA, heated in boiling water for 10 minutes, and centrifuged. Repeated the washing step using water. Dispersed the precipitate in a small volume of water and mixed with 3 mL of 1.5 N NaOH. Adjusted the total volume to approximately 30 mL with water and heated in a boiling water bath for 30 minutes.

Filtered the hot solution quantitatively through Whatman No. 1 filter paper with moderate retention. Washed the precipitate with 60-70 mL of hot water and discarded the filtrate. Dissolved the precipitate from the filter paper using 40 mL of hot 3.2 N HNO_3 into a 100 mL volumetric flask. Rinsed the filter paper with multiple portions of water, collecting the washings in the same flask. Allowed the flask and contents to cool to room temperature, then diluted to volume with water.

Transferred a 5 mL aliquot to another 100 mL volumetric flask and diluted to approximately 70 mL. Added 20 mL of 1.5 M KSCN, diluted to volume, and immediately measured the color intensity at 480 nm (within 1 minute). Determined the µg of iron present in the test using a standard curve and calculate phytate phosphorus using the given equation.

$$\text{Phytate Pmg/100g sample} = \frac{\mu g\ Fe \times 15}{\text{Weight of sample (g)}}$$

x) Statistical analysis

Antioxidant activity, phenol, flavonoid and phytate content tests experiments were performed in triplicate,

and the data are expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

1. Antioxidant activity

Metabolic processes in a living system generate free radicals, of which oxygen based ions such as singlet oxygen, hydrogen peroxide, superoxide anion are predominant. Antioxidants are essential to scavenge these free radicals in order to prevent damage in the biological system. Intrinsic antioxidants of a system may be of enzymic or non enzymic origin. Several molecules of non enzymic nature such as phenols are said to play a significant role in antioxidant activity. In order to assess the antioxidant capacity and free radical scavenging potential of the red gram genotypes, several assays such as FRAP, DPPH, ABTS, H_2O_2 radical scavenging were performed. The variety BSR 1 recorded high DPPH radical scavenging activity (49.1±0.01) and significantly high phenol content.

Red gram line ICPWSMD (25.4±0.002) recorded the highest total antioxidant activity. Several of the bioactivities observed in the red gram may be attributed to the presence of antioxidant activity. Total antioxidant activity distribution and radical scavenging potential of the red gram genotypes is given in figure 1.

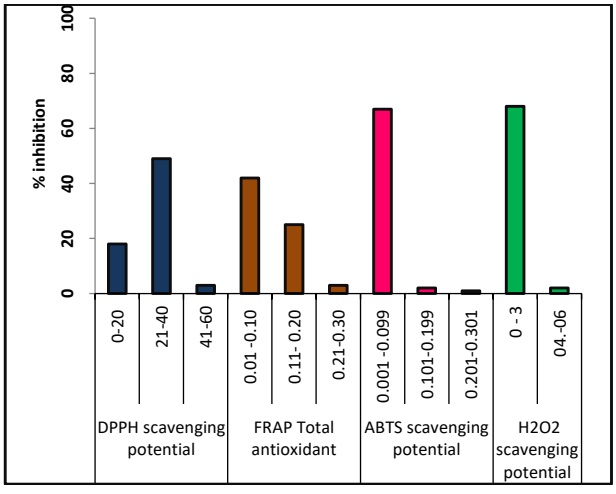

Fig. 1.Total antioxidant activity distribution and radical scavenging potential of the red gram genotypes

2.Phenols

Phenols are compounds with an aromatic ring with one or more hydroxyl groups and present in various forms such as simple phenols to complex polymerized forms. It has the ability to scavenge free radicals and interact with proteins. Phenols are significant in bestowing colour, taste and flavour to foods. High molecular weight phenolic compounds are referred as polyphenols and have complex structures. Polyphenols provide protection against oxidative stress and related damage. All these attributes make it suitable candidate in creating functional foods.

Sales *et al.,* (2014) reported that polyphenolic compounds have strong α-amylase inhibitory activity. Polyphenols not only reduce oxidative stress but also inhibit carbohydrate-hydrolyzing enzymes due to their protein-binding abilities (Ram kumar *et al.,* 2010 & Padilla *et al.*, 2014). Legumes are good sources of polyphenolic compounds like phenolic acids, flavonoids and condensed tannins, the distribution of which is more in the seed coat.

Kidney beans and mung beans contain gallic acid and protocatechuic acid, whereas catechins and procyanidins make up nearly 70% of the total phenols in the seed coat of lentils. The processing of legumes alters the chemical structure of these phenolic compounds resulting in lower antioxidant potential which is in direct relation with the chemical structure, like number and position of the hydroxyl groups. Also, water soluble phenols may leach out in cooking water. Phenolic compounds express anticarcinogenic, antithrombotic, antiulcer, antiatherogenic, antiallergenic, anti-inflammatory, antioxidant, immunomodulatory, antimicrobial, cardioprotective, and analgesic properties (Singh *et al.,* 2017). Phenolic compounds play a crucial role in cancer treatment by inducing apoptosis in cancer cells. Key and widely occurring phenols include tannins, caffeic acid, capsaicin, gallic acid, resveratrol, and curcumin. Phenols, possessing varying degrees of anticancer activity, initiate apoptosis through multiple pathways, including the extrinsic (Fas) and intrinsic mechanisms (such as calcium release, increased ROS levels, DNA degradation, and mitochondrial membrane disruption (Soltani *et al.,* 2023).

As red gram is consumed after removal of seed coat, the present study has been conducted with dehusked red gram seeds. Total phenolic content was recorded in the range of 50 to 600 mg/100g with the varieties BSR-1, BRG-1 and PT12-5-5-1 recording highest. The phenols present may contribute to the antioxidant potential of the red gram. Diversity of

pulses within and between the species is related to the variation in the content and composition of nutraceutical principles (Kumar *et al*., 2021). The distribution pattern of phenols in the red gram genotypes is presented in figure 2.

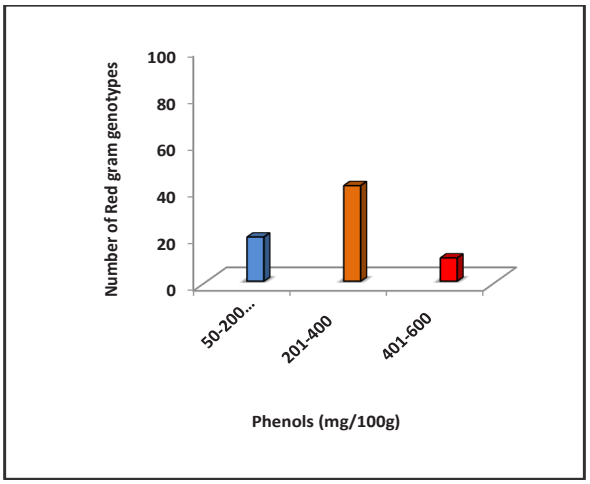

Fig. 2 Distribution pattern of phenols in the red gram genotypes

3. Flavonoid

Flavonoids are a diverse group of aromatic polyphenols primarily found in edible plants as glycosides, where they are linked to sugar molecules. The major representatives include O-glycosides of flavones and flavonols, as well as C-glycosides of flavones (Veitch *et al*., 2011). These compounds are widely distributed, low molecular weight phenolic molecules and are among the most distinctive classes of compounds found in advanced plants. They are naturally occurring in fruits, vegetables, tea, wine, and other plant based foods. Extensive research has identified various flavonoids, such as flavonols, flavones, flavanones, isoflavones, flavanols, and anthocyanins, with distinct biological properties (Wenzel, 2013).

The total flavonoid content in the red gram lines ranges between 10 to 80mg/100g. Maximum flavonoids were observed in CO8R, CO11R, ICPWSMD 2201, GJP 2011, TDRG 272, BRG-2 and BRG-4 which may contribute to antioxidant activity. Flavonoids are recognized as potent antioxidants, both *in vivo* and particularly *in vitro*, with many of their biological effects initially attributed to their radical scavenging properties (Pietta, 2000). For certain flavonoids, there is strong scientific evidence supporting their role in promoting human health. Key benefits include antioxidant activity.

Flavonoids are known for their wide range of potential health benefits. These compounds exhibit various bioactivities that are influenced by their chemical structure and interactions within the human body. Upon ingestion, flavonoids first interact with digestive enzymes and transporters in the small intestine, which play a critical role in their metabolism and absorption. One significant area of interest is their effect on glucose homeostasis. Flavonoid glycosides have been shown to inhibit enzymes such as α-amylase and α-glucosidase, which are responsible for breaking down complex carbohydrates into glucose. This inhibition slows carbohydrate digestion, potentially lowering postprandial plasma glucose levels, a beneficial effect for managing hyperglycemia in conditions like diabetes. Based on their glycosidic structure, flavonoids show potential as inhibitors of α-glucosidase, a key enzyme responsible for catalyzing the final step of carbohydrate digestion at the brush border membrane of enterocytes. For over 20 years, α-glucosidase inhibition has been clinically achieved using acarbose, a bacterial pseudo-tetrasaccharide that reduces postprandial plasma glucose levels and various cardiovascular risk factors, helping to prevent late-stage diabetic complications (Stand & Schnell, 2012). Notably, mulberry leaf extract has been found to lower postprandial hyperglycemia by inhibiting α-glucosidase without causing common acarbose related side effects such as abdominal flatulence and meteorism. This suggests that flavonoid glycosides may serve as even more effective pharmacological agents for blood glucose regulation (Kim *et al*., 2011). Flavonoid distribution in the red gram genotypes is presented in figure 3.

Flavonoids such as luteolin, myricetin, and quercetin also exhibit α-amylase inhibition as one of their mechanisms of action (Tadera *et al*., 2006 & Kim *et al*., 2000). The proposed mechanism for the inhibitory action of flavonoids on amylase activity links their potency to the number of hydroxyl groups present on the B ring of the flavonoid structure. This inhibition is attributed to the formation of hydrogen bonds between the hydroxyl groups of polyphenol ligands and the catalytic residues within the binding siteof the enzyme. Flavonols and flavones exhibit particularly high inhibitory capacities. The inhibitory activities observed varied between 100% and 50% for fisetin (Piparo *et al*., 2008).The studies highlighted differences

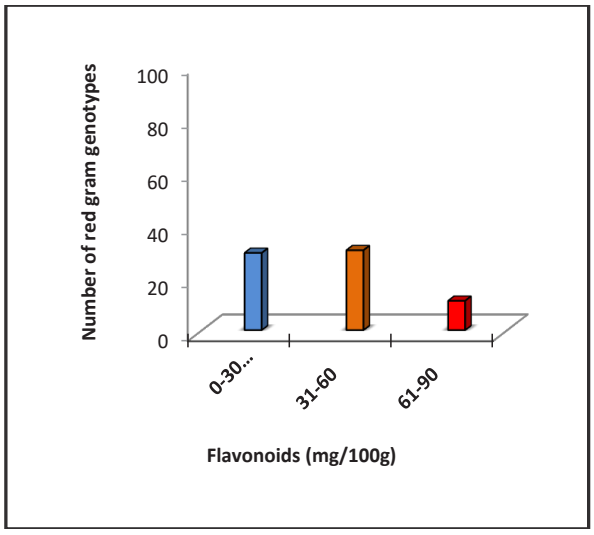

Fig.3.Flavonoid distribution in the red gram genotypes

in the concentration of the tested compounds, the incubation time of the enzyme, and the substrate solution used (Funke *et al.*, 2006 & Piparo *et al.*, 2008). These variations significantly influenced the results obtained. Variations in the percentage of inhibition were also noted for rosmarinic acid (Mentreddy *et al.,* 2007) and daidzein (Piparo *et al.,* 2008).

4. Phytic acid

Nearly 50% of the plant phosphorus is stored as phytates in the form of inositol phosphate, of which myoinositol phosphate is the most abundant one in pulses. Phytate exhibits a chelating effect due to its capacity to bind with minerals such as iron, zinc, magnesium and calcium and make it unavailable due to formation of insoluble complexes. The property of phytate to bind with proteins alter protein solubility and absorption. This impacts the activity of critical enzymes involved in digestion, such as amylase, trypsin, chymotrypsin. Not limiting to this, phytate can also bind to starch through phosphate linkages and hence affect starch digestibility. This property of phytate binding with starch, protein or minerals qualify it as an antinutritional factor. However phytate has biological significance in cellular functions like chromatin remodeling, DNA repair, hormone signaling (Zhou &Erdmn, 1995).

It also has the potential to reduce cell proliferation and tumour abrogation, implicating its role against cancer. Antioxidant activities expressed by phytate can delay glucose absorption from diet, thereby serving as potential antidiabetic candidate.

The phytate content of red gram lines ranged from 0.155 mg/g to 4.35 mg/g. The varieties CO1R, CO 2R and CO4 R recorded least phytate levels of 0.155 mg/g. Among the varieties, BSR 1 recorded the highest phytate content (0.455mg/g). The vegetable types BRG1 and BWR 23 recorded moderate phytate contents of 0.505 and 0.305 mg/g respectively. Interestingly the late lines recorded 0.23 to 0.38 mg/g. Medium duration lines accumulated phytate in the range of 0.33 to 0.355 mg/g. 19029 ICPL recorded highest phytate content of mg/g followed by 19039 ICPL and 19003 ICPL (1.8mg/g) 19037 ICPL (1.766) and 19030 ICPL (1.433mg/g). Distribution pattern of phytic acid in the red gram genotypes is presented in figure 4.

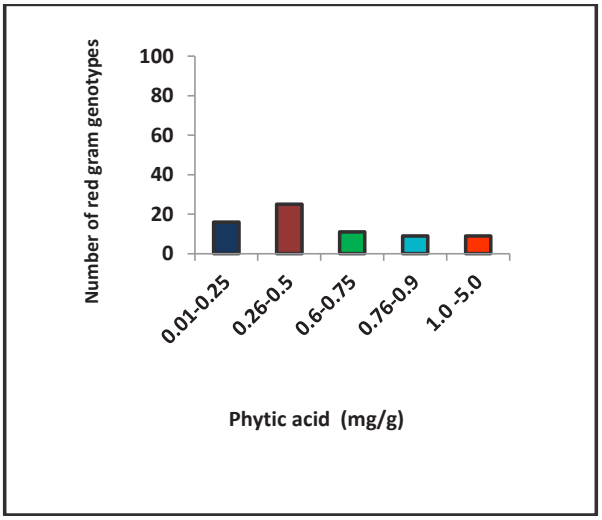

Fig. 4 Distribution pattern of phytic acid in the red gram genotypes

Phytic acid is considered an anti nutritional factor as it can complex micro and macroelements that may lead to reduction in biological role of proteins or minerals which could also be cofactors of prime enzymes. On the other hand, phytates in low concentrations can express bioactivities such as antioxidant activity, hypoglycemic effect, prevent colon cancer or fight against dental caries. The phytate content of pulses like green gram, pigeon pea, black gram, kidney bean, cow pea, lentil, soybean, chickpea and fava bean and pea varied, presenting in ranges between 3.0 to 29.3 mg/g. Among the common beans, kidney bean recorded the highest (Kumar *et al*., 2021). Dietary phytates up to 25 mg per 100g are considered to be ideal for the beneficial effects, above which there may be interference with mineral uptake

(Kumar *et al*., 2021). Pulses with higher phytates can be consumed after soaking and cooking, which can reduce the level of this antinutritional factor.

CONCLUSION

Plant-based foods play a crucial role in human nutrition and overall health. The consumption of dietary fiber, unsaturated fats, phytosterols and essential trace elements (vitamins and minerals) from plant sources is linked to improved health outcomes. These nutrients help in the prevention and management of chronic diseases such as heart disease, diabetes and obesity. Recent nutritional research has emphasized the health benefits of phytic acid and its lower inositol phosphates, including their antioxidant properties, role in diabetes prevention and anticancer effects. While the consumption of phenolic compounds is linked to numerous health benefits, it is crucial to consider the appropriate dosage for humans. However, it is important to account for the fact that these sources contain various types of polyphenols in differing concentrations. Phenols are bestowed with several nutraceutical properties *viz*., antioxidant, apoptotic, anti-aging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. This finding also encourages increasing consumption of red gram by population groups aiming at calorie reduction. The diversity in the occurrence of these bioactive principles suggest the potential role of red gram as an ingredient in the foods for specific conditions due its nutraceutical potential.

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