Essential minerals and phytic acid in legumes with reference to their nutritive and medicinal properties

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Abstract: Three commonly used legumes, *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., were subjected to determine their minerals and phytic acid contents to correlate their nutritional and medicinal properties. To quantify essential minerals, atomic absorption spectroscopic method, while for phytic acid estimation, spectrophotometric method was used. Presence of Fe, Mg, Mn, P and Zn were recorded in good quantities, Ca and Cu in moderate, while K in small quantity in the seed flours of all the three tested legumes. Maximum Fe and Zn content (0.38 and 0.40 mg/g) were recorded in *P. vulgaris,* while *M. uniflorum* delivered high content of Mg, Mn, P and Ca (0.21, 0.20, 77.94 and 0.04 mg/g) and 0.04 mg/g Cu was recorded in *P. lunatus.* The highest level of phytic acid (37.00 mg/g) was recorded in *M. uniflorum* at 519 nm. The estimated quantities of minerals and phytic acid provide a good opportunity to draw a conclusion that all the three tested legumes could potentially be used as food to achieve nutritional and health related functional benefits.

Keywords: *Macrotyloma uniflorum, Phaseolus lunatus, Phaseolus vulgaris.*

INTRODUCTION

Legumes in different geographical regions considerably differ in their use, phytoconstituents and nutritive value. Thus it may not be a very simple task to establish a common scientific correlation to justify the benefits of all the species or types of legumes in a similar way. The most popular constituents which may the basis of their usage and application in different geographical regions are linked to presence of protein and carbohydrate, followed by mineral contents and presence of soluble and insoluble fibers. The contents of these constituents may vary from region to region because of the nature of soil, climatic conditions, harvesting procedure and storage conditions, etc. The legumes, due to sizeable quantity of protein and carbohydrates, deliver reasonable caloric value when consumed as nutrients. The essential minerals present in legumes may commonly be divided into two major groups, macro minerals and micro minerals on the basis of their requirements in the human body. Interestingly, legumes are considered as one of the good sources for both groups of minerals. Three most common legumes (*Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn.,) available in Pakistan have been reviewed and reported in the present study. The scientific community treats minerals as spark plugs of human life which is composed about 4% of the human body and act as catalysts in different biochemical reactions (Bergner, 2003 ; McArdle, 2000). No biochemical mechanism exists in the human body which can produce minerals and thus all requirements must be obtained through a balanced diet. In South Asia, South East Asia and Africa, huge populations are reported to use different types of legumes for their nutritive and medicinal value. *Phaseolus lunatus* Linn. and *Phaseolus vulgaris* Linn., are reported to deliver 335 and 333 cal / 100g respectively (Akibode and Maredia, 2011), while *Macrotyloma uniflorum* (Lam.) Verdc., is reported to produce 321 cal / 100g, (Mehra and Upadhyaya, 2013) and thus is approvingly justified to be used as common and alternate economical food. In traditional recipes, the mature seeds as well as immature pods and greed seeds, all are used as food in different forms. Despite the fact that beans and legumes have been reported as rich source for essential minerals, controversial reports have also been published because of the presence of phytate (phytic acid or inositol hexaphosphate) which may or may not affect mineral absorption (Andersson *et al.*, 1983; Behall *et al.*, 1987; Sandstead, 1992; Sunvold *et al.*, 1995). Phytate mainly accumulates in protein storage vacuoles as globoids, predominantly located in the aleurone layer or in the embryo. It is the primary storage compound of phosphorus in seeds or legumes accounting for up to 80% of the total seed phosphorus and contributing as much as 1.5% to the seed dry weight. One of the reasons which may support the theory of decreased bioavailability of minerals is due to the ionic property of phytic acid which is strongly negatively charged under normal physiological conditions (Barrientos and Murthy, 1996) and thus has immense capability to form a complex (chelating conjugates) with some of the nutritionally important minerals, such as iron, zinc, magnesium and calcium

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which are positively charged multivalent cations (Cosgrove, 1980). Although the resulting complexes are soluble in acidic environment of stomach but get precipitated in the intestinal pH (very weakly acidic to neutral) resulting in poor absorption of minerals and trace elements. It is also reported that once the complex is formed, the effect of phytic acid may not be reversed even in the presence of the enzyme, phytase. However, some exogenous and endogenous chelating agents (e.g., ascorbic acid and high molecular weight mucoprotein respectively) have the capability to compete with phytic acid-mineral complex, thus helping in releasing minerals at the site of absorption (mucosal cell surface or within the cell). This may reverse the negative impact of phytic acid through increased bioavailability (Dintzis, 1989 ; Erdman and Poneros-Schneier, 1989; Jacobs and Miles, 1969; Teucher *et al.*, 2004).

The exogenous and endogenous chelating agents may increase the bioavailability of minerals by dissociating the phytic acid-mineral complex or by forming a soluble complex with minerals which may compete and inhibit the formation of phytic acid-mineral complex.

The negative impact of soluble fibers (phytates) on mineral absorption have been widely studied in adults and a conclusion was drawn that some dietary components, such as fermentable carbohydrates, organic acids produced during fermentation of food and the content and type of proteins can counteract the inhibitory effects of phytates on mineral absorption (Coudray *et al.*, 1997 ;Lönnerdal *et al.*, 1989; Lönnerdal, 2000; Lopez *et al.*, 2000: Pabón and Lönnerdal, 1992; Rimbach *et al.*, 1995 ;Sandström *et al.*, 1989; Trinidad *et al.*, 1996). Very few studies in infants have been reported regarding effects on mineral absorption. Iron absorption is greatly affected by phytic acid both in adults and infants, however, its influence on zinc absorption in infants seems to be modest (Davidsson *et al.*, 1994; Hurrell, 2003; Hurrell *et al.*, 1998).

M. uniflorum, *P. lunatus* and *P. vulgaris* belong to family Papilionaceae. Apart from Pakistan, *M. uniflorum* is also available in Africa, Australia, Bhutan, India, Indonesia, Myanmar, Nepal**,** Philippine, and Sri-Lanka. The seeds are light or deep reddish brown in color with orbicular-reniform in shape (Wu *et al.*, 2010). *P. lunatus* is native to tropical America. The seeds are brown, red, purple, and black in color with Sub-rhombic reniform in shape. *P. vulgaris*is also native to tropical America with dark red seed of reniform shape (Nasir and Ali, 1977). Both, *P. lunatus* and *P. vulgaris* are freely available in Pakistan.

The traditional system of medicine which is commonly practiced in Indo-Pak subcontinent recommends the seeds of *M. uniflorum* to treat a large number of diseases, such as diarrhea, hepatomegaly, splenomegaly, dysuria, leucorrhoea, obesity, asthma, bronchitis, hiccup, colic and it also promotes lochia discharge (Alok *et al.*, 2014). As scientific evidences in terms of clinical trials are lacking, therefore, apparently, it will not be an easy task to justify all the usages and applications as recommended in traditional system of medicines. With regard to pharmacological activity, seeds are reported to possess astringent, anthelmintic, antipyretic, anti-cholelithiatic (Bigoniya *et al.*, 2014), anti-diabetic(Gupta *et al.*, 2011), anti-hepatotoxic, anti-peptic ulcer, litholytic (Brink and Belay, 2006), antioxidant, leading to anti-carcinogen (Siddhuraju and Manian, 2007), diuretic (Alok *et al.,* 2014) larvicidal (Gupta *et al.*, 2011), α-amylase and angiotensin-I converting enzyme inhibitory activities (Sreerama *et al.*, 2012a). Beans of *Phaseoluslunatus* Linn. Exhibited hypolipidemic (Oboh and Omofoma, 2008), succinate dehydrogenase, acid phosphatase, α-glucosidase and tyrosine-kinase inhibition activities (Aniszewski, 2006 ; Yao *et al.*, 2011) while, *P. vulgaris* exhibited anti-inflammatory and antioxidant activities (Oomah *et al.*, 2010).Based on these interesting findings, the present study was undertaken to evaluate the three most popular legumes *M. uniflorum*, *P. lunatus* and *P. vulgaris* available and commonly consumed in Pakistan to quantify their nutrient (minerals) and anti-nutrient (phytic acid) components and to correlate their merits and demerits for the human body. The word anti-nutrient for phytic acid has been suggested by a number of researchers based on its property to bind minerals (chelating property) in the GIT, thus reducing their bioavailability. However, at the same time, based on its chelating property, a number of researchers have used phytic acid (Inositol hexaphosphate $InsP_6$) successfully to prevent renal stone formation (Henneman *et al.*, 1958 ; Ohkawa *et al.*, 1984 ; Sakamoto *et al.*, 1993). Authors of the present research article have also tried to highlight some of the positive effects of phytic acids of legumes, such as antioxidant, anti-cancer, litholytic effects along with their effects on cholesterol and blood sugar. In addition, methods of preparation have also been highlighted how to reduce the content of phytic acid before consumption, if the purpose is to get more benefits of nutrients and not of the anti-nutrient.

MATERIALS AND METHODS

Plant material identification and sample preparation

The seeds of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., were purchased from a local market and identified by taxonomists in the Department of Botany, University of Karachi. The voucher specimen number of *M. uniflorum* (G.H.No.86483), *P. lunatus* (G.H.No.86451) and *P. vulgaris* (G.H.No.86536) were deposited in the herbarium of University of Karachi Pakistan. Seeds were separately grinded into fine powder and then finally passed through 600μm sieve and kept in an amber colored bottle at room temperature before studies were commenced.

Etimation of minerals

Preparation of samples

Concentrated HNO_3 (1.0 ml) was added to each sample (1.0 g) in a small beaker. Beakers were allowed to stand overnight followed by reflex heating on a water bath under a controlled exhaust system till the production of red nitrous oxide fumes ceased. The resulting solutions were allowed to cool at room temperature. To the content of each beaker, 4.0 ml of 70% $HClO_4$ was added and the mixtures were further heated to reduce the volume. The solutions were then filtered and the volume in each beaker was adjusted to 50 ml with distilled water. The resulting solutions were then used to estimate calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K) and zinc (Zn) (Elmer, 1996).

Preparation of standard solutions and standard curve

Standard solutions (1000 ppm) of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P) and zinc (Zn) were obtained from the Fisher Scientific-UK. A standard curve was developed using serial dilutions of 20, 40, 60 ppb prepared by diluting 1000 ppm standard solutions with different diluents using volumetric flasks of 0.01% accuracy. Freshly prepared stock solution of each mineral was used to avoid degradation. Additionally, all glass ware, apparatus and disposable tips of micropipette were cleaned and rinsed with deionized double distilled water prior to use. Atomic absorption spectrophotometer (GF-AAS) linked with Perkin Elmer A Analyst-700 was used to determine minerals. The concentration of minerals was directly obtained from the standard curve and all measurements were performed in triplicate for both standard and sample solutions.

Etimation of phytic acid

The standard curves of phytic acid, concentrations ranging from 0.5-5% were prepared by measuring the absorbance at 519 nm against distilled water as control.

Extraction of phytic acid

1g of each bean sample was added to 10 ml of 5 % H_2SO_4 solution with continuous stirring and the pH was adjusted to 0.4-0.5. The mixture was then shaken for 30 mins at room temperature and finally centrifuged at 4000 rpm for 30mins at room temperature (Saad *et al.*, 2011).

Estimation method

The method reported by Saad *et al.,* 2011 was used with some modification. Supernatant (1.0 ml) as obtained from extraction was added to ferric (III) chloride solution (1.0ml) and the solution was heated on water bath at 100°C for 30 minutes. The resulting solution was treated with ice cool water for 15mins and centrifuged at 4000 rpm for 30 minutes at room temperature. The supernatant (1.0ml) was transferred to cuvettes and few drops of 2, 2' bipyridine solution were carefully added till the appearance of light pink color. The absorbance of reaction mixture was immediately measured at 519nm (Saad *et al.,* 2011) against distilled water as control. Blank was run parallel against the sample. The sample readings were calibrated with the standard curve of phytic acid for each set of analysis.

STATISTICAL ANALYSIS

Levels of essential minerals and phytic acid were subjected unpaired student's *t*-test. All statistical calculations were performed with SPSS-20.

RESULTS

The results of the mineral analysis (mg/g) obtained from all the three tested beans have been depicted in table 1 along with reported RDA limit of a particular mineral in order to compare the content with the estimated values. The phytic acid content (mg/g) was determined at 519nm and calculated using the standard curve. Phytic acid was found as 37.0 in *M. uniflorum*, 30.3 in *P. lunatus* and 33.6 in *P. vulgaris*.

DISCUSSION

Estimated mineral contents were also compared with the available USDA (United States Department of Agriculture) nutrient index data relating to nutritional composition of *P. lunatus* and *P. vulgaris*. Composition of *M. uniflorum* is not presentin USDA nutrient index. The estimated values of the micro and macro-minerals from all tested flour were significant (*P* □0.01) with the exception of Mn from *P. lunatus*. The sample of *M. uniflorum* was noted to be superior to *P. lunatus* and *P. vulgaris* with respect to the content of Ca, Mg and Mn. *P. lunatus* was observed to be superior to *M. uniflorum* and *P. vulgaris* while estimating Cu and K contents, whereas *P. vulgaris* was recorded better than *M. uniflorum* and *P. lunatus* with respect to content of Fe and Zn. Results of the present study extended valuable support to the theory that seed flours of all the three legumes tested were noted as good sources of Fe, Mg, Mn and Zn and moderate sources of Ca, Cu and K.

In earlier studies (Sreerama *et al.*, 2008 ; Sreerama *et al.*, 2010 ; Sreerama *et al.*, 2012b), phytic acid content ranging from 1.02 to 10.20 mg/g have been reported, whereas, in the present study, we observed a higher phytic acid content, especially in *M. uniflorum*. The difference in phytic acid content may be accredited to a reduction of phytic acid content upon long storage intervals. The long storage period of legumes decreases phytic acid content in legumes and cereals, both depending on the storage conditions (especially humidity and temperature) and the type of seeds and the age of the seeds (Martín-Cabrejas *et al.*, 1997; Schlemmer *et al.*, 2009). Temperature plays an important role in the enzymatic hydrolysis due to the

Table 1: Mineral contents (mg/g) of *Macrotyloma uniflorum, Phaseolus vulgaris* and *Phaseolus lunatus* reported by USDA, RDA with their current estimated values and calculated value on the basis of daily consumption.

Minerals	*Macrotyloma uniflorum*		*Phaseolus lunatus*		*Phaseolus vulgaris*		RDA§	Calculated value (mg) on the basis of daily consumption					
								Macrotyloma uniflorum		*Phaseolus lunatus*		*Phaseolus vulgaris*	
	USDA report	Estimated value	USDA report¶	Estimated value	USDA report††	Estimated value		100g	200g	100g	200g	100g	200g
Ca	NR†	0.04±0.0002*	0.81	0.03±0.0003*	0.83	0.03±0.0005*	1000–1300 mg/day (for adults)	4.2	8.4	3.4	6.8	3.0	6.0
Cu		0.03± 0.0011*	NR†	0.04±0.0004*	NR†	0.02±0.0011*	3mg/day (for adults)	3.5	7.0	3.7	7.4	2.4	4.8
Fe		0.33± 0.0006*	0.06	0.40±0.0004*	0.07	0.38±0.0034*	8mg/day (males); 8-18mg/day (females)	32.7	65.4	37.1	74.2	37.9	75.8
K		0.30±2.5612*	14.03	0.70±1.5937*	13.60	0.52±6.7823*	3500mg/day(for adults)	29.7	59.4	66.4	132.8	52.12	104.24
Mg		0.21±0.0011*	1.90	0.21±0.0003*	1.4	0.19±0.0012*	420mg/day(males); 320mg/day(females)	21.3	42.6	21.2	42.4	19.2	38.4
Mn		0.20±0.0010*	NR†	0.10±0.0009	NR†	0.13±0.0002*	2.3 mg/day (males); 1.8mg/day (females)	17.6	35.2	9.7	19.4	13.3	26.6
P		77.94± 0.004*	3.70	ND‡	4.06	75.54±0.006*	--------	7794.3	15588.6	ND‡	ND‡	7554	15108
Zn		0.34±0.0005*	0.03	0.23±0.0017*	0.03	0.40±0.0025*	11mg/day(males) 8mg/day(females)	33.7	67.4	22.6	45.2	39.7	79.4

†NR=Not reported; ‡ND=Not determined; §RDA adopted from (Mohammed and Ahmad, 2014); ¶*Phaseolus lunatus* (USDA, 2014b); ††*Phaseolus vulgaris* (USDA, 2014a). All values has been roundup accordingly. N=5 for each group (results are mean of five determination on a dry weight basis ± S.E.M.); S.E.M.=Standard Error of Mean; *P□0.01 showing significant values using unpaired student's *t*-test.

increased formation of phytase (phosphohydrolase) with the passage of time (Lei and Porres, 2003). However, if kept under controlled conditions (below 45°C; less than 4.5 or more than 6.0 pH) phytase remains inactive.

Essential minerals have been reported to be involved in various biochemical reactions in the human body which finally result in the synthesis of a number of important biomolecules required in the body to perform certain critical functions. In addition, minerals are also involved in some specific physico-mechanical processes which allow the body to maintain its integrity and to perform specific functions. Therefore, a balanced diet is the best solution for obtaining the required quantity of the essential minerals and allows the body to regulate various biochemical reactions smoothly. Deficiency of essential minerals will certainly lead to the development of certain diseases or syndromes. Consequently, demanding the body to fulfill the requirements from external source, such as through ready to use mineral supplements in different dosage forms. Calcium is mainly stored in bones and thus occupies a prominent position in performing some essential functions in the body, such as contraction of muscles, expansion and constriction of blood vessels, regulation of nervous system, and secretion of certain hormones and enzymes which ultimately regulates certain important biochemical reactions or processes. Redox enzymes are essential for human body and copper is the key component of redox enzymes, while manganese can function as a component of some minerallo-enzymes. Similarly, one of the major roles documented for iron is in the synthesis of hemoglobin and myoglobin. Zinc has been declared as an essential component which has significant involvement in the development of sex organs and their function, thus its deficiency may affect fertility to a great extent, while growth retardation, weakness of muscles as well as improper cardiac functions have been linked with magnesium deficiency (Mohammed and Ahmad, 2014). The results of the quantification of minerals support the usefulness of *M. uniflorum*, *P. lunatus* and *P. vulgaris* seed flour for better health when compared with recommended daily allowances of essential minerals as proposed by international standard organizations.

The correlations between the various constituents of legumes, especially the presence of both soluble and insoluble fibers and the beneficial effects have been extensively highlighted in various reports. Most studies suggest that the soluble fibers in legumes, such as pectin, gums, mucilage, and some hemicelluloses can help in maintaining the blood sugar balanced due to very low glycemic index (GI) which ultimately reduces the demand of insulin secretion. High insulin secretion reported to play important role in the development of type 2 diabetes mellitus (Willett *et al.*, 2002). It is further reported that replacement of saturated fats and refined carbohydrates with legumes may have protective effects can reduce the risk of cardiovascular disease, including decrease in total serum cholesterol, LDL, triglyceride and increase in HDL – cholesterol (Anderson and Major, 2002 ; Mitchell *et al.*, 2009). It is further reported that high plasma homocysteine levels are coupled with enhanced cardiovascular disease risk, such as coronary heart disease, stroke and peripheral vascular disease. Homocysteine may elevate atherosclerosis by injuring the inner lining of arteries and promoting blood clotting. As legumes are classified under good sources of folic acid, it helps to lower homocysteine level. In addition, legumes are also documented as good sources of magnesium and potassium, which may decrease cardiovascular disease risk by regulating blood pressure (Anderson and Major, 2002). In the present study, high quantity of magnesium and potassium as recorded in all the three tested legumes can be linked to their beneficial effects in managing cardiovascular diseases.

The insoluble fibers of legumes which include cellulose, lignin and some hemicellulose are not absorbed and thus pass through the digestive tract without any change. These fibers have been reported to provide two most important benefits which include reduction in the risk of hemorrhoids and constipation along with improving the function of colon. In spite of these facts, some individuals do not feel comfortable and avoid consuming legumes mainly because of the excess formation of intestinal gas. However, this unwanted effect can be minimized by soaking the beans in water preferably for few hours or overnight before cooking. The soaking will remove some of the gas-causing substances, after which the beans can be easily digested.

Seeds of *M. uniflorum* (Das *et al.*, 2005) and *P. vulgaris* (Duke, 2008) are reported to possess litholytic activity to a greater extent because of their potassium and magnesium contents as well as of phytic acid content which may be helpful in preventing the formation of stones. In the present study the potassium content in all the three beans was recorded sufficient to correlate their usefulness. The content of magnesium and phytic acid were recorded in good quantities, justifying litholytic activity. It is evident from various reports that regular intake of potassium or magnesium above the recommended concentration, suppresses the formation of stones because potassium promotes urinary citrate excretion and together with magnesium it further inhibits crystal formation. Alkali therapy using potassium or magnesium citrate is reported to boost citrate excretion in calcium oxalate-stone formers with hypocitraturia (Johri *et al.*, 2010). Magnesium is also an inhibitor of urinary stone. It can compete with calcium for oxalate and thus form complexes with oxalate with the formation of magnesium oxalate which is more soluble than calcium oxalate. Thus, oral intake of magnesium will decrease the oxalate absorption and urinary excretion in the form of calcium oxalate because of the formation of magnesium oxalate complex and will subsequently decrease the super saturation of calcium oxalate. It is now evident that magnesium supplementation in subjects with magnesium deficiency increases the excretion of magnesium citrate in urine (Basavaraj *et al.*, 2007). Therefore, increase in urinary magnesium citrate excretion, after magnesium supplementation suggests that magnesium has suppressive role in renal stone formation, through its effect on citrate metabolism. It is further reported that magnesium ion has the property to destabilize calcium oxalate ion pairs and thus reduce the size of the aggregates. The magnesium ion's inhibitory effect is synergistic with citrate and remains effective in acidic environments (Reungjui *et al.*, 2002). However, the rate limiting factor is the absorption of ingested magnesium in human body, which is between 20 to 50% (McCarthy and Kumar, 1999).

In case of calcium phosphate stone, potassium citrate therapy is recommended in view of its inhibitory effect on calcium phosphate crystallization. Due to electrolyte imbalance issue, potassium citrate is preferred over sodium citrate (Pearle *et al.*, 2014). The content of potassium and magnesium in these legumes supports the theory with the suggestion that a regular intake of these legumes will extend prophylactic mechanism and will effectively be involved in reduction of stone formation. Studies also suggest that potassium citrate based medical prophylaxis is effective in preventing stone recurrence regardless of previous treatment modalities, stone composition, metabolic abnormalities and stone-free status. Based on some earlier studies on prophylactic mechanism, it is expected that a regular intake of these legumes may also be helpful to people with known history of multiple stone recurrence, calcium oxalate di-hydrate stones, hypercalciurea and hyperuricosuria (Lee *et al.*, 1999). Further, as potassium citrate treatment is also useful in the management of uric acid lithiasis (Pak *et al.*, 1986) therefore a possible role of these legumes does exist in this condition as well.

In terms of medicinal application and importance, phytic acid plays an important role to inhibit the crystallization of oxalate and phosphate salts of calcium (Grases and Costa-Bauza, 1999). Phytic acid inhibits the intra papillary tissue and urinary (renal tubular and vascular) crystallization by inhibiting precipitation of Brushite (calcium hydrogen phosphate di-hydrate - $CaHPO_4{\cdot}2H_2O$.) and Whewellite (calcium oxalate monohydrate - $CaC_2O_4{\cdot}H_2O$). In normal concentration (0.3-0.4μM), phytic acid has been reported to inhibit the development of dystrophic calcification (Schlemmer *et al.,* 2009). The availability of phytic acid in tissues and blood depends on dietary intake and these levels correlate with that of urinary excretion (Grases *et al.*, 2007). The litholytic role of phytic acid is enhanced by its ability to bind with calcium to reduce its bioavailability (Kirk-Othmer, 1994), and its antioxidant action (Selvam, 2002). Cytotoxic substances with oxidative capacity and hyperoxaluria induces renal tubular cell injury by the production of free radicals due to lipid per oxidation in proximal tubules (Huang *et al.*, 2003). Renal epithelial cell injuries in renal papilla invites Whewellite to form attached renal calculi at the sites and cause the development of Whewellite papillary calculi. Antioxidant activity plays an important role in prevention of these calculi formation (Grases *et al.*, 2009). Other beneficial activities of phytic acid include lowering of blood glucose levels, cholesterol and LDL. Potential anti-cancer behavior has also been reported by phytic acid in several studies demonstrating the usefulness of high-fiber diets in reducing colon cancer risks (Shamsuddin, 2002).The overall reported biological and physiological activities include strong antioxidant, anti-inflammatory, immune function enhancer, phase I and II metabolizing enzymes modifier, oncogene expression modulator, cell proliferation normalizer, cell differentiation and apoptosis inducer, and angiogenesis inhibitor (Vucenik and Shamsuddin, 2006). Although, the strong chelating property of phytic acid with minerals and trace mineral reduces their bioavailability, a balanced diet has been reported to cause less or insignificant problem with respect to bioavailability (Schlemmer*et al.,* 2009). The oral LD_{50} values for phytic acid are reported as 405 and 500 mg/kg body weight/day in male and female rats whereas 900 and 1,150 mg/kg body weight in male and female mice. Published human studies indicated 1000 to 2000 mg/day phytic acid consumption along with balanced diet without affecting mineral status. Negative impact on mineral absorption has been observed in human only when phytic acid is consumed in very large quantities with poor mineral diets (unbalanced diet) especially in developing countries (USDA, 2012). However, such negative effects are not as imminent in developed countries because of balanced diet consumption. Assuming a daily consumption of 100 to 200 g of the legumes tested in the present study are expected to provide around 3700 to 7400 mg/day of phytic acid based on the highest concentration of phytic acid (37.0 mg/g) as determined in *M. uniflorum.* Therefore, based on various reports and studies published elsewhere, it is expected that the high concentration of phytic acid present in the tested legumes may interfere the absorption of minerals affecting bioavailability as well. However, this may need further in depth investigations and studies before reaching a firm conclusion with regard to interference in bioavailability of minerals with the use of these tested legumes due to high phytic acid content. Further, the purpose of use of legumes will also have a great impact in achieving the desired effect. It is expected, that high concentration of phytic acid in these tested legumes will impose greater medicinal value, such as litholytic, antioxidant, anticancer and some other beneficial activities, including lowering of blood glucose levels and cholesterol even if we assume its negative impact on the bioavailability of minerals upon its daily consumption. The possible reasons for the detection of high phytic acid in the tested legumes has already been proposed and reviewed during early part of the discussion.

While exploring and correlating the effect of high phytic acid content in tested legumes in absorption of minerals, some interesting analytical results have been seen which are required to be addressed here. One of the most important findings of the present study is the detection of high iron and zinc content along with the high phytic acid content, whereas at the same time potassium content was noted to be significantly lower than the USDA data (USDA, 2014a, USDA, 2014b). It is really difficult to interpret the actual reason for high iron and zinc along with low potassium content in the tested legumes. One of the reason of high iron and zinc content in the seeds, recently been proposed by the authors of a study conducted in Poland (Głowacka *et al.*, 2015), using *Phaseolus vulgaris* is the method of weed control and weather in different growing seasons. Presence of weeds and sufficient rainfall greatly affect iron and zinc content in the legumes. Many weeds are valuable soil conditioners whose vigorous roots penetrate deeply into the subsoil, transporting nutrients and minerals to the surface. This improves the soil structure through aeration and control of erosion, while sufficient rainfall may make the entire soil more fertile and favorable for cultivation and yielding of the crops, finally resulting in accumulation of high mineral contents in the seeds. As for the low potassium content, it can be linked with the genetic diversity and environmental conditions as reported in some studies. The values of potassium in these studies range from 4.42g/kg to 24.80g/kg while investigating mineral contents of *Phaseolus vulgaris* (Barampama and Simard, 1993; Moraghan and Grafton, 2002; Mesquita *et al.*, 2007; Poersch *et al.*, 2011). Results of present study indicate maximum level of potassium as 0.7g/kg. This value is still higher than the lowest values reported in earlier studies, but lower than the USDA value which is 14.03mg/g for *Phaseolus lunatus* (USDA, 2014a, USDA, 2014b).

The iron content of various bean seeds is reported to range from 40.00mg/kg to 98.43 mg/kg, whereas, quantity of zinc has been reported from 17.7mg/kg to 65.32mg/ kg (Blair *et al.*, 2009;Głowacka *et al.,* 2015; Meyer *et al.*, 2013). In the present study, highest iron content was recorded in *Phaseolus lunatus* as 0.4mg/g or 400mg/kg, and highest zinc content in *Phaseolus vulgaris* as 0.4mg/g or 400mg/kg. These quantities are slightly higher than the quantities mentioned in USDA reports (USDA, 2014a, USDA, 2014b), but still lower than the quantities in above studies. Apparently, the high content of iron and zinc present in tested legumes may be helpful in authenticating the amount of iron and zinc required for absorption, even in presence of high phytic acid content which is capable of reducing bioavailability of these micronutrients. This proposed reason is based on one of the facts that iron absorption can be improved by increasing the iron content of the diet by fortification or supplementation (Whittaker, 1998), while other two facts include, increasing dietary factors that enhance iron absorption, such as vitamin C, or by decreasing factors that inhibit iron absorption, such as phytic acid. Interestingly, perhaps nothing is required to be added or eliminated while consuming these legumes because high iron and zinc contents, in presence of high phytic acid logically support the first factor, i.e., iron absorption can be increased by increasing the iron content of the diet. Therefore, high iron and zinc content in the tested legumes may be treated as natural fortification or supplementation. As all the three components (iron, zinc and phytic acid) are present naturally in one product (legume), this may be regarded as a balanced combination and thus can be used to achieve both nutritive as well as medicinal effect or can also be consumed for maintaining a healthy life through its prophylactic effects. Further, in view of WHO declaration (Carvalho *et al.*, 2012), that iron deficiency is one of the leading and most common factors for malnutrition in developing countries, the consumption of these legumes may be helpful in alleviating malnutrition as well as in improving the quality of life which is a major public health concern. The overall information and data presented in the present study not only supports the beneficial effects of phytic acid present in the tested legumes but also advocates that it should not be treated as an anti-nutrient.

CONCLUSION

Human body has a complex mechanism to accept or reject the contents of various foods consumed by an individual for a particular purpose. In most cases the nutrients and anti-nutrients are reported to be present together and may have different functions once they enter the human system. The mineral contents of the legumes along with both soluble and insoluble fibers are evaluated in the present study and their usage and application is justified from both modern and traditional concepts. However, the purpose, quantity and preparation of legumes may have significant impact upon its benefits. As human physiology varies considerably between individuals, therefore even the two individuals with similar consumption pattern of legumes, may behave differently with respect to bioavailability of minerals due to the presence of phytates.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this paper.

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