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HPLC ANALYSIS OF BIOACTIVE COMPOUNDS IN TEN DIFFERENT WILD TYPE UNDER-UTILIZED LEGUME GRAINS

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ABSTRACT

In recent years, many food industries have been initiated the formulation of nutraceutical/functional foods by incorporating the bioactive ingredients for the prevention/treatment of certain chronic diseases. In this connection, certain promising wild type under-utilized legume grains received more attention, since they are naturally a rich source of L-Dopa (precursor of dopamine) and certain bioactive compounds including phenolics, tannins and phytic acid. In the present study, seed materials of certain promising wild type under-utilized food legume grains such as* Abrus precatorius *L.,* Acacia leucopholea *Willd,* Bauhinia varigata *L.,* Canavalia gladiata *(Jacq.) DC.,* Cassia floribunda *Cav.,* Entada scandens *Benth.,* Indigofera linifolia *(L.f.) Retz.,* Mucuna monosperma *DC. Ex Wight.,* Sesbania bispinosa *(Jacq.) Wight. and* Tamarindus indica *L., collected from Eastern and Western Ghats of South India, were investigated for certain bioactive compounds through HPLC technique. All the analysed samples were found to constitute a viable source of total free phenolics (4.23 – 8.75 g/100 g DM), tannins (1.04 – 5.41 g /100 g DM), L-Dopa (1.17 – 5.34 g/100 g DM) and phytic acid (0.96 – 2.74 g/100 g DM) and also the newly developed HPLC procedures were proven to be sensitive enough to detect these bioactive compounds even at tracer level. Further, such wild type legume grains could be recommended as a natural source of bioactive compounds in the dietary management of certain chronic diseases such as Parkinsonism, diabetes, obesity, cardiovascular diseases, cancer etc.

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Keywords: *Wild type legume grains; bioactive compounds; total free phenolics; tannins; L-Dopa; phytic acid*

[I] INTRODUCTION

Legume grains have been playing a key role in the traditional diets of human beings throughout the world. They are excellent source of protein, dietary fibre, starch, micronutrients and bioactive compounds with low level of fat. The total per capita consumption of legume grains has been increased markedly over the past two decades in US, due to increased attention to beans being classified as functional foods [1]. Accumulation of chemical, biochemical, clinical and epidemiological evidences indicating a positive correlation between the consumption of legume seeds and decreasing incidence of several chronic diseases such as cancer, cardiovascular diseases, obesity and diabetes [2]. Such obvious health benefits of legume seeds are attributed to presence of certain bioactive compounds such as phenolic acids, flavonoids and tannins [3]. Therefore, at present the studies on bioactive compounds, which are responsible for health promoting/disease preventing effect, are being increased in addition to the evaluation of nutritive profiles of legume grains.

Actually in olden days, the bioactive compounds like total free phenolics, tannins, L-Dopa and phytic acid were considered as antinutritional substances and their presence in food/feed-stuffs was reported to be undesirable from the nutritional point of view. But, now-a-days, the health beneficial role of such bioactive compounds has been explored by a large number of research studies. Particularly, these bioactive compounds were demonstrated to possess many favourable medicinal properties, including potential antioxidant activity [3,4,5]. As a consequence of health beneficial effects, presence of such bioactive compounds in the diet has been viewed in a positive light in recent years by both scientists and consumers and resulted in a push to procure foods with specific health benefits such as functional foods.

Apart from common legume seeds, the earlier research efforts revealed the nutritive potential of certain promising under-utilized/wild legume seeds, including the pulses of tribal

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utility. Among the various under-utilized legumes, the seed materials of *Abrus precatorius* L., *Acacia leucopholea* Willd, *Bauhinia varigata* L., *Canavalia gladiata* (Jacq.) DC., *Cassia floribunda* Cav., *Entada scandens* Benth., *Indigofera linifolia* (L.f.) Retz., *Mucuna monosperma* DC. Ex Wight., *Sesbania bispinosa* (Jacq.) Wight. and *Tamarindus indica* L. merit a wider use as a food legume. Their distribution, agronomic traits, nutritional value, mode of consumption was described in detail by Janardhanan *et al*. [6]. In South India, these wild type legume grains are being traditionally consumed by certain ethnic groups, particularly the Kanikkar, Lambadi, Uraali and Dravidian tribes living in Tamilnadu, Kerala, Karnataka and Andhrapradesh States.

Even though, few reports are available regarding the nutritional value of the above-mentioned under-utilized legume grains, information on bioactive compounds are very meagre and also no standard HPLC methods were reported for the analysis of total free phenolics, tannins, L-Dopa and phytic acid. Hence, a very sensitive and reliable HPLC technique should be developed in order to detect these compounds, before using them in the dietary management trials of various oxidative-stress related diseases. So that, we can easily measure these compounds in the biological samples like blood plasma, urine, etc., more accurately within short period of time. In the present study, an attempt has been made to develop suitable HPLC methodologies to analyze the levels of bioactive compounds of ten different under-utilized legume seeds collected from Eastern and Western Ghats of South India.

[II] MATERIALS AND METHODS

2.1. Chemicals

Poly-vinyl-polypyrrolidone, (+)-catechin hydrate, vanillin, tannic acid, L-Dopa, phytic acid were procured from Sigma-Aldrich Chemicals, USA; Sephadex LH-20 was obtained from Pharmacia Fine Chemicals, Sweden; Anion exchange resin was purchased from Bio-Rad, USA, and all other chemicals and HPLC grade solvents were received from Merck, Darmstadt, Germany.

2.2. Collection of seed samples

The details on collection of seed materials of wild type legume grains from different agro-climatic locations of Eastern and Western Ghats of South India were given in Table 1. After removing the immature and damaged seeds, the mature seeds were dried under shaded condition for two days. All the samples were freezed at -80°C and freeze-dried for 48 h. Then the samples were first cracked with the help of a wooden hammer into small pieces and subsequently powdered in a seed mill (Siemens, Germany) to 1 mm particle size, freeze-dried for 24 h and stored at 9°C until further use.

2.3. Total free phenolics

The total free phenolics were extracted from seed samples by taking 1 g of defatted seed flour sequentially with 10 ml of 100%, 80% and 50% methanol and 70% acetone acidified with 1% conc. HCl in an ultra-sonic bath (Bandelin Sonorex, RK – 514 H, Berlin, Germany) for 30 min. After centrifugation, all the supernatants were collected and made up to a known volume. The extract was treated with 500 mg of poly-vinyl-polypyrrolidone at 0°C for 30 min. Then the contents were purified by using a Solid Phase Catridge (SPC) (Strata-x-33 um polymeric sorbent, L100-1105, 200 mg/6 ml sample, 8B-S100-FCH-S from Phenomenex, USA). Then the solvents were evaporated in a rotary-vacuum evaporator (Büchi Rotavapor – R, CH-9230, Switzerland) at 40°C and 25 mbar pressure and dried in lyophilier (Virtis Freeze-mobile – 25 EL, New York). The contents were re-dissolved in 1 ml of solvent (Methanol:water:formic acid, 47.5:47.5:5%) and transferred to a HPLC vial. The HPLC apparatus consists of Varian Prostar-210 High pressure gradient pump, Waters-2487 Dual absorption detector, Triathlon autosampler (Spark Holland, The Netherlands), operated by Galaxy Chromatography Data Systems (GCDS), version-1.9.3.2 from Varian. The column consists of Reprosil-Pur 120 C18 AQ 5 µm (250 x 4.6 mm size) column from Trentec, 71273 Rutesheim, Germany, which is maintained at 40°C. Solvent A (water and formic acid, 95:5%, v/v) and solvent B (water, acetonitril and formic acid, 85:10:5%, v/v/v) were used as mobile phase with the gradient of 20% B at 0 min, 32% B at 6 min, 63% B at 6.01 min, 84% B at 11 min, 100% B at 11.01 min100% B at 15 min, 20% B at 15.01 min and 20% B at 20 min. Flow rate of 1 ml/min was used and the signals were detected at 280 nm. Based on the standard curve prepared with (+)-Catechin hydrate (20-100 µg), the amount of total free phenolics in the extract was calculated and expressed in g/100 g seed flour on dry matter basis.

Name of the wild type legume grain	Colour of the seed coat	Place of collection
Abrus precatorius	Red & black	Guruvayur, Kerala State
Acacia leucopholea	Black	Bhavanisagar, Tamilnadu State
Bauhinia varigata	Light brown	Mysore, Karnataka State
Canavalia gladiata	Light brown	Guntur, Andhrapradesh State
Cassia floribunda	Reddish brown	Kodivery, Tamilnadu State
Entada scandens	Dark brown	Tirunelveli, Tamilnadu State
Indigofera linifolia	Grey	Kollegal, Karnataka State
Mucuna monosperma	Light brown	Thiruvalla, Kerala State
Sesbania bispinosa	Dark green	Kadambur, Tamilnadu State
Tamarindus indica	Dark brown	Kanyakumari, Tamilnadu State

Table: 1. Data on collection of seed materials of certain wild type legumes collected from different agro-ecological locations of South India.

2.4. Tannins

The tannins were extracted from the seed materials by taking 1 g of defatted seed flour sequentially with 100%, 90%, 80% and 70% acetone solutions acidified with 1% conc. HCl. After centrifugation, all the supernatants were pooled together and made up to a known volume with acetone. Then the extract was purified by using Sephadex LH-20 column chromatography (96 x 1.6 cm) with acetone:water (50:50, v/v) as a solvent [2]. After collecting 20 fractions (5 ml each), the active fractions were identified and pooled together, evaporated and lyophilized as described above and used for analysis. For HPLC/PDA analysis, 20 µl was injected into a Varian HPLC (Pro Star

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210) equipped with a Shimadzu PDA (PD-M20A) applying the following chromatographic conditions: Reprosil-Pur 120 C18 AQ column (5µm, 250 x 4.6 mm) at 40°C, and solvent A of aqueous formic acid (5% v/v) and solvent B containing formic acid, destilled water and acetonitril (5:10:85, v/v/v). The gradient program was as follows: 5% B to 26% B (12.38 min), 26% B to 100% B (17.22 min), 100% B isocratic (10 min) with a flow-rate of 1 ml/min and a total run time of 40 min. and the signals were detected at 280 nm. The standard curve was prepared by taking different concentrations of tannic acid and the level of tannins was calculated.

2.5. L-Dopa

Finely ground seed flour (1 g) was treated with 10 ml of petroleum ether and kept in an ultra-sonic bath for 30 min. Then the defatted pellet was extracted with 10 ml of 0.1 N HCl. The contents were vortexed for 10 min at room temperature (25°C) and kept in an ultra-sonic bath for 30 min under ice bath condition and subsequently it was kept on a magnetic stirrer for 1 h at room temperature. The supernatant was collected by centrifugation (13,000 x g, 15 min) and the extraction procedure was repeated twice and all the supernatants were pooled and diluted to a final known volume and used for further analysis. Then the extract was evaporated and lyophilized and re-dissolved in water the ratio of 5 mg extract/ml. The same HPLC system described above was used with the eluting solution (Solvent A: water, methanol and phosphoric acid in the ratio of 975.5:19.5:1, v/v/v, pH 2.0), and washing solution (Solvent B: 70% methanol). The gradient used was: start with 100% (A) and 0% (B) up to 12 min, next 5 min solvent (B) increase from 0 to 100%, decrease B to 0% in the next 5 min, and then the column is washed with solvent- A alone in the next 15 min to adjust the column to the starting conditions. Isocratic elution was carried out and the separation was performed at room temperature (25^{o}C) at the flow rate of 1 ml/min and the signals were detected at 282 nm. The L-Dopa content was calculated based on the standard curve prepared with synthetic L-Dopa.

2.6. Phytic acid

The phytic acid was extracted from raw and differentially processed seed samples by taking 1 mg of defatted seed flour with 10 ml of 2.4% HCl and incubated for 10 min in ultra-sonic bath. Then the contents were centrifuged at 13,000 x g for 5 min and the supernatant was collected. Similarly, the residue was re-extracted twice and all the supernatants were pooled together and made up to a known volume with distilled water. The extract was purified by using an anionic-exchange column chromatography (0.7 cm x 15 cm) containing 0.5 g of anion-exchange resin (100–200 mesh, chloride form; AG1-X4, Bio-Rad Co., CA, USA). The phytic acid was eluted with 2 M HCl and used for quantification. The aliquot was analyzed on a Merck-Hitachi HPLC (LaChrom) equipped with a column oven (set at 40°C), fluorescence detector (L-7480) and Clarity chromatographic station (DA-C50, DataApex Ltd, Praha). The separation was achieved on a 5 µm analytical column (Grom-Sil 120 ODS-4 HE, 125 x 4 mm, Grom, Rottenburg-Hailfingen, Germany) using a mobile phase consisting of methanol (27.5% v/v) and phosphate buffer (pH 7.0) at a flow rate of 0.8 ml/min. Phytic acid was detected by excitation/emission set at 367/435 nm.

2.7. Statistical analysis

All the data were analyzed and expressed as means ± standard deviation of five separate determinations (n = 5). The statistical analysis was carried out by using SPSS for Windows (SPSS Inc., Chicago, IL, version 11.0). Values of analyzed compounds were found to be normal distributed by using Kolmogorov-Smirnov-test. Means of the groups were compared by one-way ANOVA and Dunnett post-hoc test using the raw seeds as a control. Two-tailed P values < 0.05 were considered statistically significant.

[III] RESULTS AND DISCUSSION

3.1. Total free phenolics

The phenolic compounds constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, which are ranged from simple molecules (eg. phenolic acids, phenyl-propanoids and flavonoids) to highly polymerized compounds (eg. lignins and melanins). Now-a-days, the phenolic compounds are demonstrated to prevent the development of many chronic diseases such as atherosclerosis, diabetes, cancer *etc*. Such protective effect of phenolics might be associated with their powerful antioxidant and free radical scavenging properties [7]. The seed coat of legume grains are reported to contain numerous types of phenolics, which playing an important protective role against oxidative damage in consumer's body [2].

The total free phenolics content of raw seed materials of different wild legume grains were found to range between 4.23 and 8.75 g/100 g seed flour DM **[Figure-1]**. These values are higher when compared to the previous reports on broad bean (2.39 g/100 g DM); pea (2.26 – 3.48 g/100 g DM); white bean (1.08 g/100 g DM); black bean (4.40 g/100 g DM) and common bean (1.88 – 2.53 g/100 g DM), but comparable with that of faba bean (5.59 g/100 g DM); Adzuki bean (8.97 g/100 g DM); red bean (5.54 – 9.36 g/100 g DM); red lentil (5.80 g/100 g DM); green lentil (6.76 g/100 g DM) and brown bean (9.14 g/100 g DM) [8].

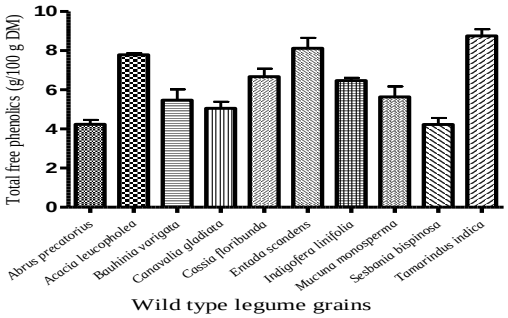

Fig: 1. Total free phenolics content of ten different wild type legume grains

In general, the total free phenolics content of presently investigated under-utilized legume grains were appears to be higher when compared to the literature [6]. This might be due to the repeated extraction of phenolic compounds by using both methanol and acetone as solvents as well as sensitivity of HPLC method **[Figure-2]**. Recovery of phenolic compounds from legume grains is mainly depends upon the type of solvent used and the duration of extraction. Acetone and methanol extracts of seed samples exhibited higher phenolic yield when compared to either methanol or acetone used alone [9].

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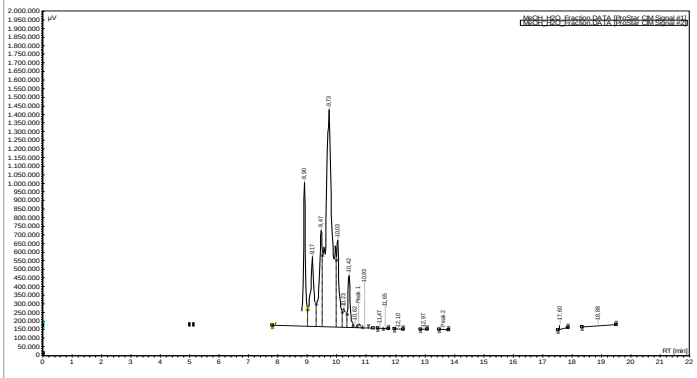

Fig: 2. HPLC analysis of total free phenolics in wild type legume grains.

The seed samples of *Tamarindus indica* (8.75 g/100 g DM) registered significantly ($p < 0.05$) higher level of total free phenolics, which is followed by *Entada scandens* (8.12 g/100 g DM) and *Acacia leucopholea* (7.79 g/100 g DM) **[Figure-3]**. It is interesting to notice that the seed coat colour of these seed materials is dark brown/black. Relationships between seed coat colour and phenolics level are still controversial. While Barampama and Simard [10] found a positive relationship between the seed coat colour and phenolic content, Guzman-Maldonado *et al*. [11] did not find any relationship. However, there are some reports available with high correlation between cultivar lines and phenolic content [12]. In addition to seed coat colour, it is well documented that the quantity of phenolic compounds in seed samples is influenced by soil, environmental conditions, genotype (cultivar/variety), agronomic practices (irrigation, fertilization and pest management), maturity level at harvest and post-harvest storage. For instance, low temperature during the onset and duration of seed fill were shown to increase the isoflavone content by several folds in soybean [13]. Since these under-utilized legumes grow wildly in adverse environmental conditions such as drought, poor soil *etc*., a high phenolic content contributes to the resistant function.

Although the dietary intake of phenolics varies considerably among the geographical regions, it is estimated that the daily intake of total free phenolics was ranged from 20 mg to 1 g, which is higher than the intake of vitamin E. Hence, in recent years, food technologists are keen to harness the nutritional benefits of phenolics, namely its antioxidant or free radical scavenging, food preservative, antimicrobial, anti-mutagenic, therapeutic and pharmaceutical properties.

3.2. Tannins

Beside simple phenolics mainly found in cellular vacuoles, some polymerized form of phenolics with varying degree of solubility such as tannins are also noticed in legume seeds. Tannins are defined as a unique group of phenolic metabolites of relatively high molecular weight. Concerning chemical structure, they can be divided into four groups: condensed tannins, hydrolysable tannins, phlorotannins and complex tannins [14]. Tannins possess ideal structural chemistry for better free radical scavenging activity and hence, they exhibit more effective antioxidant activity under *in vitro* conditions than tocopherols and ascorbic acid [15]. The free radical scavenging power of tannins is closely connected with their spatial confirmation and degree of polymerization. Further, both the hydrolysable and condensed tannins are demonstrated to possess more effective and greater antioxidant activity than simple phenolics.

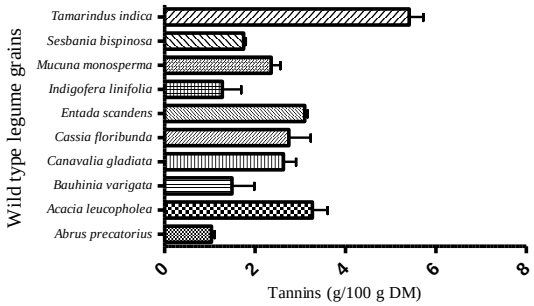

Fig: 3. Tannins content of ten different wild type legume grains.

The tannins content of raw under-utilized legume grains were found to falls between 1.04 and 5.41 g/100 g DM **[Figure-3]**. These values are found to be higher when compared to previous reports on green pea (0.003 – 0.17 g/100 g DM); yellow pea (0.15 g/100 g DM); chickpea (0.18 g/100 g DM); lentil (0.012 – 0.88 g/100 g DM); red kidney bean (0.012 – 0.55 g/100 g DM); black bean (0.04 – 0.67 g/100 g DM) and common bean (0.02 – 0.13 g/100 g DM) [8]. Such a high level of tannins in wild legume seeds when compared to the literature [6] might be due to the type of solvent used for extraction as well as accuracy of the presently developed HPLC technique **[Figure-4]**. Similarly, Chavan *et al*. [16] and Troszyska *et al*. [2] reported the maximization of extraction of tannins from beach pea and yellow pea seed coats, respectively, when acetone was used as a solvent compared to methanol.

It is noticeable that, the seed samples with dark brown coloured seed coat like *Tamarindus indica* (5.41 g/100 g DM) and *Entada scandens* (3.10 g/100 g DM) as well as black coloured seed coat *Acacia leucopholea* (3.27 g/100 g DM) were registered significantly ($p < 0.05$) higher level of tannins than the other seeds.

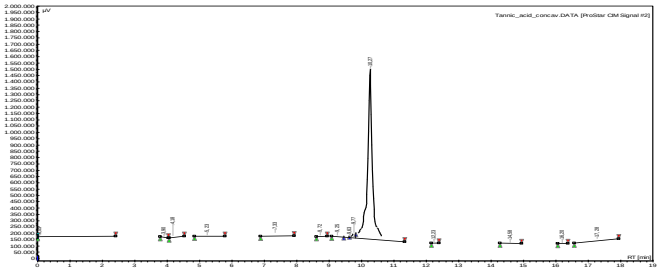

Fig: 4. HPLC analysis of tannin in wild type legume grains

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It is postulated that high level of condensed tannins or proanthocyanidin are seen in dark coloured beans than in yellow or white coloured beans. Since, the level of phenolics was relatively low in pale coloured seeds; it is possible to assume that the major phenolics in dark coloured coated seeds could be proanthocyanidins. Recent studies have demonstrated a quantitative pattern of heredity for tannins content and that tannins level is also associated with seed coat colour inheritance.

Several factors, such as plant type, cultivar, age of the plant or plant parts, stage of development and environmental conditions were reported to govern the tannins content in legume grains. Presence of high content of tannins in the presently studied wild legume seeds might be due to the metabolism of polyphenolic compounds or polymerization of existing phenolic compounds during development or maturation [16]. According to Serrano *et al*. [14], the mean daily intake of condensed tannins among US population (>2 year old) was estimated to be 53.6 mg/person/day, whereas 450 mg/person/day in the Spanish diet. There are a lot of epidemiological data, which suggested that tannins intake may prevent the onset of many chronic diseases. The positive biological effects including antioxidant, anticarcinogenic, anti-mutagenic, antimicrobial, antiviral and anti-diabetic properties of tannins have been extensively studied.

3.3. L-Dopa

L-Dopa (L-3,4-Dihxdroxyphenylalanine) is a non-protein phenolic amino acid, mainly used in the treatment of Parkinson's disease, since it is the precursor of dopamine. L-Dopa has also been investigated as a dietary supplement to manage hypertension, renal failure and liver cirrhosis. Further, the protective effects of L-Dopa on small bowel injury, ulcer, gastro-intestinal diseases, diabetes as well as antioxidant stress were scientifically proved by earlier studies [17]. The seed materials of wild legume grains, especially the *Mucuna* sp. was reported to contain appreciable level of L-Dopa [18].

The raw seed materials of different wild legume grains of the present study recorded the L-Dopa content of 1.17 - 5.34 g/100 g DM **[Figure-5]**. These values are found to be comparable with that of certain under-utilized legumes such as *Cassia floribunda* (1.57 g/100 g DM); *C. obtusifolia* (1.34 g/100 g DM); *Canavalia ensiformis* (2.64 g/100 g DM) and *C. gladiata* (2.83 g/100 g DM) [19], but, lower than that of *Mucuna cochichinensis* (6.11 g/100 g DM) and *M. veracruz* (7.12 g/100 g DM) [20]. The presently developed HPLC method is found to be more sensitive **[Figure-6]**, but has a drawback that the compound should be analyzed within 8 h of extraction to avoid the oxidation of L-Dopa.

The L-Dopa content varies considerably at significant level ($p < 0.05$) among the wild legumes of the present investigation. The seed samples of *Mucuna monosperma* recorded the maximum level of L-Dopa content (5.34 g/100 g DM), while the low level was observed in *Tamarindus indica*.

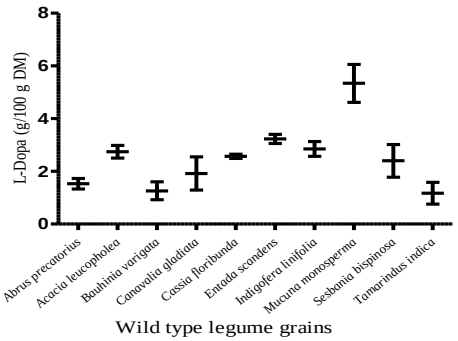

Fig: 5. L-Dopa content of ten different wild type legume grains.

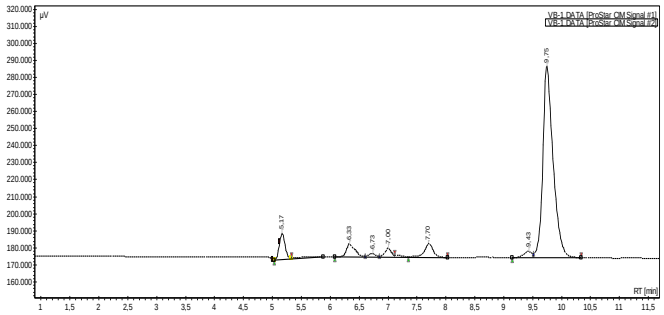

Fig: 6. HPLC analysis of L- Dopa in wild type legume grains

In general, the *Mucuna* species is naturally a potential source of L-Dopa and commercially used for the extraction of this compound for the treatment of Parkinsonism. Such a wide variability in L-Dopa content among wild legumes could be cause by both environmental effect and genetic nature. For instance, presence of more L-Dopa was noticed in the *Mucuna* plants growing near the equator (within 10°) than the plants cultivated far away from equatorial regions in earlier investigations. Further, the L-Dopa synthesis is reported to be high in plants growing at low latitudes, near the equator [18]. It was also hypothesized that variation in the intensity of light and backscattered ultraviolet radiation, both generally more near the equator, may be among the factors explaining why the L-Dopa content was found to be high in plants growing at low latitudes.

3.4. Phytic acid

Phytic acid (myo-inositol hexaphosphate) is widely found in cereals, nuts, legumes, oil seeds, pollen and spores, constituting about 1–5% and generally the legume seeds are regarded as the major source of dietary phytate [4]. In recent years, the phytic acid is considered as an antioxidant, anti-carcinogenic, hypoglycemic and hypolipidemic agent, in addition to the fact that a high phytate diet can be effectively used in the treatment of hyper-calciuria and kidney stones in human beings [21]. The wild type legume seeds were found to contain 0.96 - 2.74 g/100 g DM of phytic acid **[Figure-7]**.

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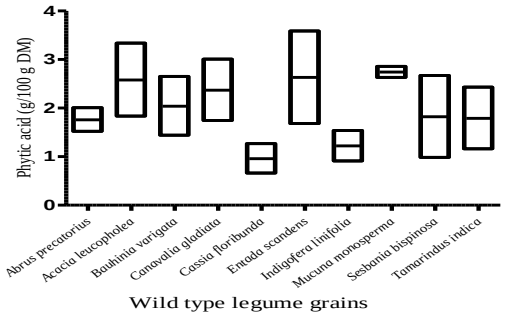

Fig: 7. Phytic acid content of ten different wild type legume grains.

These values are comparable with that of an earlier report on *Phaseolus vulgaris* (0.61 – 2.38 g/100 g DM); *Vicia faba* (0.51 – 1.77 g/100 g DM); *Pisum sativum* (0.22 – 1.22 g/100 g DM); *Vigna unguiculata* (0.37 – 2.90 g/100 g DM); *Cicer arietinum* (0.28 – 1.60 g/100 g DM) and *Lens culinaris* (0.27 – 1.51 g/100 g DM) [21].

The HPLC chromatogram revealed the presence of two different fractions (tetra- and penta-phosphate myoinositol) of phytic acid **[Figure-8]**. Considerable level of variation on the phytic acid content of presently investigated under-utilized legume grains might be attributed to both genetic and environmental conditions. In general, the cultivar, which contains appreciably high amount of protein is observed to be The estimated daily phytic acid intake of human population was about 750 mg in U.S.A.; 600 - 800 mg/day in U.K.; 393 mg/day among Canadian children; 2000 - 2200 mg/day in Nigeria; 1890 and 569 mg/day in Malawi and New Guinea, respectively and 1487 mg/day in East India [21]. But, historically it has been considered as an antinutrient and postulated to impede the bioavailability of minerals. Nevertheless, the research studies conducted by Grases *et al*. [23] showed that there is no negative effect on the mineral balance and element bioavailability due to the oral administration of phytic acid, even in the second generation rats.

[IV] CONCLUSION

The HPLC techniques developed to analyze the bioactive compounds of under-utilized legume samples were found to be more appropriate and sensitive enough to detect the total free phenolics, tannins, L-Dopa and phytic acid even at tracer levels. Ten different wild type unconventional legume seeds collected from various agro-ecological regions of South India were found to constitute rich source of bioactive compounds. Among the under-utilized legume grains, significantly high level of total free phenolics and tannins were noticed in *Tamarindus indica*, *Entada scandens* and *Acacia leucopholea* seeds, while *Mucuna monosperma* registered maximum level of L-Dopa and phytic acid. Hence, presently studied wild type legume seeds could be recommended in order to increase the dietary intake of health beneficial bioactive compounds. Such associated with high phytic acid content. Hence, as the protein content increases, the phytate level is also found to increase in the seed samples. Al-Numair *et al*. [22] reported that the amount of phytic acid is always exceeds than that of phosphorus for all the legume cultivars, which indicates that the ratio would be more than 100%. Generally, in legume seeds, the phytic acid level is positively correlated with total phosphorous, correlation coefficients being greater than 0.90. Factors that affect the total phosphorous content, such as soil, available phosphorous and fertilizer can also influence the phytic acid concentration.

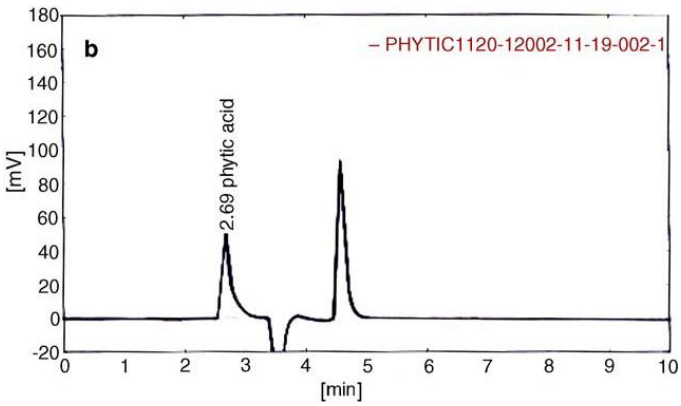

Fig. 8. HPLC analysis of phytic acid in wild type legume grains

promising under-utilized legume seeds could be explored in the dietary management of certain chronic diseases such as diabetes, obesity, cancer, cardiovascular diseases, *etc*., after conducting bioavailability studies with suitable *in vivo* model. Identification of phenolic fractions of the wild legume grains is in progress with LC-MS technique.

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