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ANTIOXIDANT POTENTIAL OF DIVERSE INDIAN CULTIVARS OF LENTILS (*LENS CULINARIS* L.)

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ABSTRACT

Antioxidant activity was evaluated in six Indian cultivars of lentil (Lens culanaris L.) viz. L-4076, L-4594, L-4147, DPL 15, DPL 62, and IPL 406. Three solvents, 70% ethanol, 100% methanol and 80% acetone, were used for preparation of seed extract. The seed extract was tested for total phenolic content, total flavonoid content and antioxidant activity by FRAP assay. The results showed that values for TPC, TFC and FRAP assay were greatly influenced by the type of organic solvent used and the genotype. The total phenolic content ranged from 272 -14.7 mg GAE/g, total flavonoid content 7.57-0.72 mg QE/g and FRAP 9.32-0.79 mMol Fe^{2+}/g. Amongst all the cultivars tested, DPL-62 and IPL - 406 exhibited high phenolic content as well as antioxidant activity which were higher than previously reported for lentils. The most efficient solvent for total phenolic extraction and antioxidant activity evaluation was 80% acetone, whereas 100% methanol and 80% acetone both showed similar results for flavonoid extraction. The results of the present study conclude that Indian cultivars of lentils possess significant amount of phenolics and antioxidant activity that may be potentially beneficial for health.

KEY WORDS

Lentils, phenolic content, flavonoid content, antioxidant activity, FRAP.

INTRODUCTION

Food legumes, commonly known as pulses, are gaining interest in the area of developing healthy and functional foods. Pulses are good source of proteins, carbohydrates, vitamins, minerals, omega -3 fatty acids as well as important health promoting non-nutrient bioactive compounds including non-starch polysaccharides, phytosterols, saponins, isoflavones, a class of phytoestrogens, phenolic compounds and antioxidants such as tocopherols and flavonoids [1,2]. It is very likely that bioactive components work in concert to promote health [3]. Consumption of pulses have been shown to be associated with beneficial effect on the prevention and management of obesity [4], coronary heart disease [5], diabetes [6], and the metabolic syndrome [7]. The DASH (Dietary Approaches to Stop Hypertension) diet for those with high blood pressure [8], as well as the gluten free diet for the people who suffer from Celiac disease [9], also include pulses as significant components of their diet [10]. The consumption of pulses is increasing globally in view of their high nutritional value, low in calories and glycemic index as well as cholesterol lowering effect [11]. Among pulses, lentils are gaining popularity not only in India but globally also. Lentil is a cool season food legume which constitutes one of the most important dietary components in Indian cuisine. They have been classified among soft seed coated pulses that

require shorter cooking time and thus have smaller losses in nutrients as compared to those with hard seed coat [12]. The phenolic compounds present in lentils can be characterized into phenolic acids, flavanols, flavonols, soyasaponins, phytic acid and condensed tannins [13]. Some phytochemicals were thought to be antinutritional factors in the past like tannins, phytic acid because of their protein binding properties [14]. Studies suggest that phenolic compounds present in lentils confer protection against chronic diseases through a multitude of biological activities including antioxidant, anticancer, angiotensin I-converting enzyme inhibition, reducing blood lipid and reducing the risk of cardiovascular diseases [15]. Recently, it has been found to be associated with reduction in incidence of HDL cholesterol and type-2 diabetes, activating innate defense mechanism and managing obesity [16]. However, unlike other legumes such as soybean, the phytochemical profiles of lentils and their potential health benefits have not been well studied [17].This explains the increasing interest among different research groups for characterization of phytochemical content and antioxidant activity in lentil [18-22]. The present study was undertaken to compare the Total Phenolic content (TPC), Total flavonoid content (TFC) and antioxidant activity in Indian cultivars of lentil after extraction from raw seeds using different extraction solvents.

MATERIALS AND METHODS

Lentil Samples:

Three Lentil varieties, L-4076, L-4594, and L-4147 were obtained from Pulses Research Lab, IARI New Delhi. Other three varieties, DPL 15, DPL 62, and IPL 406 were obtained from Indian Institute of Pulses Research Kanpur. They were stored at 4˚c until use.

Reagents for analysis:

Folin Ciocalteu's reagent, Gallic acid, Quercetin, TPTZ (2,4,6-Tripyridyl-s-Triazine) were obtained from Sigma-Aldrich, USA. All other reagents and chemicals used in the present study were of analytical grade.

Methods:

Preparation of seed extracts of lentil:

Mature dry lentil seeds were ground to pass mesh sieve to obtain a fine powder. Seed flour was extracted with different solvents viz. 70% ethanol, 100% methanol and 80% acetone according to the method [19] with slight modification. Briefly, 0.2g of powder was extracted in 2 ml microfuge tube with 2ml of solvent. The mixture was shaken at 300 rpm at ambient temperature on an orbital shaker for 3 hours. The mixture was extracted for an additional 12 hours in dark overnight. The extracts were centrifuged by Remi cooling centrifuge- 412-LAG at 12000 rpm for 15 minutes and the supernatants were collected in fresh tubes. Extracts were stored at 4˚c until further analysis within 2 days. The experiment was performed in triplicates.

Determination of Total phenolic content (TPC) of crude seed extract:

The extracts were analyzed for total phenol content using the Folin- Ciocalteu method as followed by [23, 24]. Seed extract 50µL, distilled water 3mL, Folin Ciocalteu's reagent 250 µL and 7% Na_2CO_3 (750 µL) was vortexed and incubated for 8 min at room temperature. Then, 950µL of distilled water was added. This mixture was allowed to stand for 2 hours at room temperature. The absorbance was measured at 765nm against distilled water as a blank. Calibration curve of gallic acid was drawn having a linearity range of 50 to 2500 µg/mL. The experiment was performed in triplicates. Total phenol content was expressed as mg of gallic acid

equivalents (GAE)/g of sample and was calculated using following formula:

$$\frac{\text{mg of phenol in assay} \times 1000\ \mu L \times \text{total dilution}}{\text{Vol. of sample in assay 1 mL of sample}} = \text{mg GAE/g}$$

Determination of Total flavonoid content:

Flavonoid content was determined using Aluminium chloride colorimetric method as followed by [25]. To 500µl extract 1.5 ml solvent was added followed by 100µl Aluminium Chloride, 100µl potassium acetate and 2.5 ml distilled water. The mixture was kept at RT for 30 minutes and absorbance was measured at 415nm. The calibration curve was obtained by preparing quercetin solutions at concentrations 6.25 to 180 µg/ml in 70% ethanol, 100% methanol and 80% acetone separately. TFC was expressed as mg quercetin equivalents/g of dry sample and was calculated using the above mentioned formula.

Determination of Antioxidant activity:

Antioxidant activity was determined using Ferric Reducing Antioxidant Power Assay (FRAP) as followed by [26]. Following reagents were prepared: Acetate Buffer (300 mM, pH 3.6), 40 mM HCl, 10 mM TPTZ and 20 mM Ferric Chloride. FRAP reagent was prepared as a mix of 900 ml acetate buffer (300mM pH 3.6), 90ml TPTZ (10 mM), 90ml ferric chloride (20 mM) in ratio 10:1:1, v:v:v. Solution was mixed properly by keeping in water bath at 37˚c and made fresh on the day of assay.

To 150 µL extract 4.5mL FRAP reagent was added. Absorbance was read at 593nm after 5min. The blank consisted of FRAP reagent. The final absorbance of each sample was compared with those obtained from the standard curve made from ferric sulfate heptahydrate (1-5 mM/L). Results were expressed in mMol Fe^{2+}/g of seed sample.

Statistical Analysis:

Analysis for antioxidant activity as well as for TPC and TFC was done in triplicates. The average value and standard deviation were calculated using the Microsoft excel software. T test (unpaired) was carried out to test any significant differences between different cultivars and between the solvents used. Significant levels were defined using $P<0.05$

RESULTS AND DISCUSSION

Total phenolic content:

Total phenolic content of lentil seeds as estimated by Folin-Ciocalteu's method varied significantly ($P < 0.05$) with the type of extraction solvent used as well as the genotype (Table 1). DPL-62 showed the highest phenolic content (272.0 mg GAE/g) in 80% acetone whereas L-4147 showed the lowest phenolic content (14.7 mg GAE/g) in 100% methanol. The TPC of all the selected cultivars as extracted by different solvents ranged from 53.1 to 205.1 mg GAE/g for DPL-15; from 48.6 to 272 mg GAE/g for DPL-62; from 38.7 to 232.2 mg GAE/g for IPL-406; from 34.5 to 185.4 mg GAE/g for IPL-4147; from 33.2 to 172.4 mg GAE/g for L-4046 and from 22.9 to 149.7mg GAE/g for L-4594.

The TPC extracted by the selected solvents were in following order from high to low 80% acetone> 70% ethanol> 100% methanol for all the cultivars. The results suggest that 80% acetone gave the highest yield among three solvents for extraction of total phenolics (Fig. 1).

The mean values for total phenolic content of the lentil crude extract of the selected Indian cultivars was higher than those reported earlier for green lentil [19-22, 27]. A higher level was found, however, in fraction 2 of red lentil acetone extract as separated by column chromatography [28], which may be due to concentration of all the phenolics in the purified fraction. The difference may also be due to the genotypes of lentils and

different extraction methods. Lentils have been reported exhibit higher phenolic content as compared to other legumes like peas, yellow peas, chickpeas, soybean red kidney bean and black beans [19]. As reported earlier, acetone has been the preferred solvent for extraction of phenolics [19-20, 22, 27, 28].

Total flavonoid content:

Flavonoids are wide spread plant secondary metabolites. In order to estimate the potential role of flavonoids on antioxidant activity of lentil cultivars, total flavonoid content of the extracts were analyzed. Seed extracts from different lentil cultivars extracted using different solvents differed significantly in their TFC ($P<0.05$). The TFC of DPL-15 ranged from 0.79 to 5.25 mg QE/g; DPL-16 from 1.19 to 4.14 mg QE/g; IPL-406 from 0.72 to 4.12 mg QE/g; L-4594 from 1.05 to 4.91 mg QE/g; L-4076 from 0.83 to 3.95 mg QE/g and L-4147 from 1.32 to 4.89 mg QE/g (Table 2). The results showed that DPL-15 gave the highest yield of flavonoids (7.57 mg QE/g) in 100% methanol and IPL-406 gave the lowest yield (0.72 mg QE/g) in 70% ethanol. The TFC yields by the extraction solvents were in the following order from high to low: 100% methanol > 80% acetone > 70% ethanol for DPL-15, DPL-62, and L-4076 and 80% acetone >100%methanol >70% ethanol for IPL-406, L-4594 and L-4147. The results suggest that 80% acetone was best among three solvents for extracting flavonoids from IPL-406, L-4594 and L-4147, whereas 100% methanol was the best for extracting total flavonoids from DPL-15, DPL-62, and L-4076. 70% ethanol gave the lowest yield in all the cultivars (Table 2, Fig. 2).

The mean TFC values reported earlier in American cultivars were lower [19] than the tested Indian lentil cultivars. However, [20] reported higher TFC values which may be attributed to difference in method adopted for extract preparation, extraction solvent as well as genotype of the lentil. The total flavonoid content of Indian lentils was also higher than other legumes peas, chickpea, soybean, red kidney bean and black bean [19].

Ferric Reducing Antioxidant Power:

The phenolic compounds from crude seed extracts of selected lentil cultivars exhibited ferric reducing power in FRAP assay which was monitored at 593 nm. The antioxidant concentrations of the extracts varied significantly ($P<0.05$) with the genotype and type of extraction solvent used (Table 3). The antioxidant concentration of L-4594 ranged from 1.03 to 9.32 mM; L-4076 from 0.9 to 9.22 mM; DPL-15 from 0.79 to 9.22 mM; L-4147 from 2.01 to 9.22 mM; IPL-406 from 0.9 to 9.22 mM and DPL-62 from 1.08 to 9.22 mM. Results showed that DPL-15 showed lowest activity (0.79 mM) in 100% methanol and L-4594 showed highest activity (9.32 mM) in 80% acetone. Antioxidant activity of crude acetone extracts did not show much variation (Fig. 3). The antioxidant concentration was affected by the extracting solvents in the following order from high to low: 80% acetone> 70 % ethanol> 100% methanol for all the cultivars. These results suggested that 80% acetone was the best for phenolic extraction and FRAP antioxidant assay. Mean FRAP value of crude extracts of selected lentil cultivars was slightly lower than that obtained in American cultivars extracted in 70% acidic acetone [19]. Higher FRAP values for lentils have been reported in ethanol extracts [21] which may be attributed to the extraction solvent or genotype of lentils. Protein fractions of Indian cultivars of red as well as black lentils have also been assessed for FRAP assay [29] where black lentils had significantly higher activity than red lentils. As compared to other legumes, Indian lentils possess higher antioxidant activity than peas, chickpea, yellow soybean, red kidney bean but lower than black soybean and black bean [19].

Fig. 1 Total phenolics (mg GAE/g) of different lentil cultivars as affected by different solvents

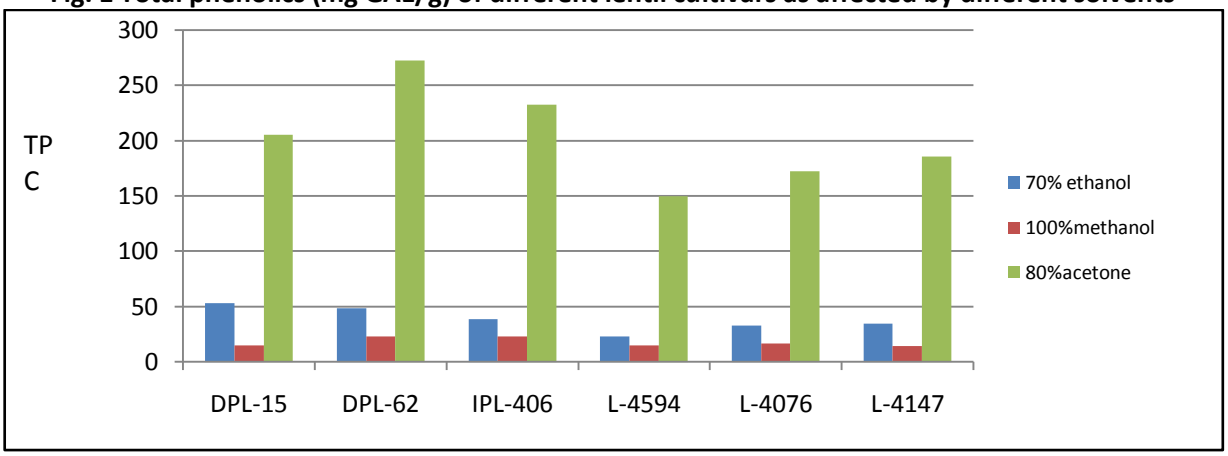


Fig. 2: Total Flavonoid Content (mg QE/g) of different lentil cultivars as affected by different solvents.

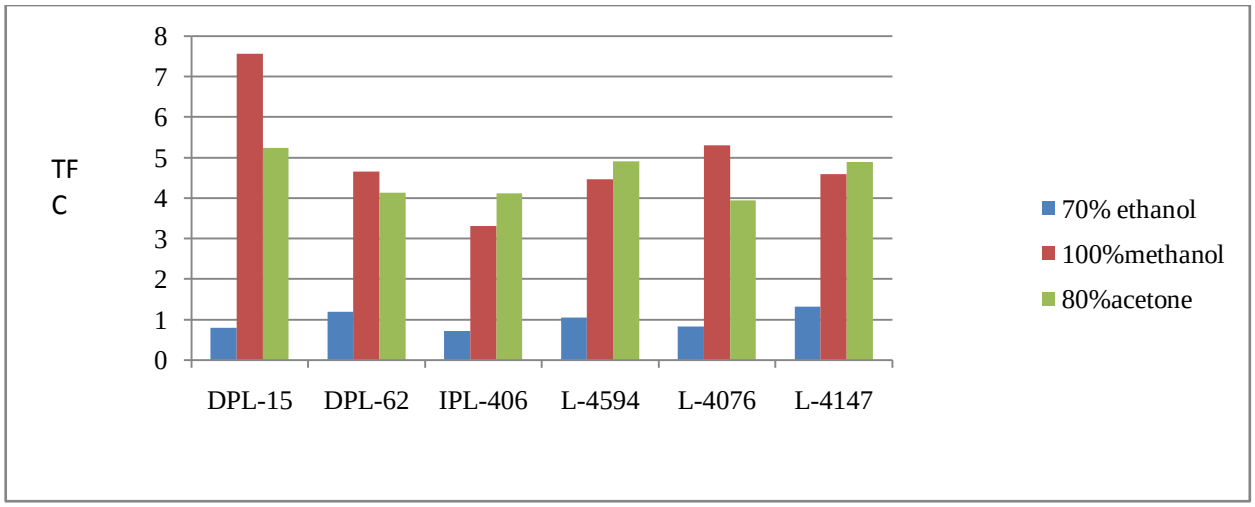
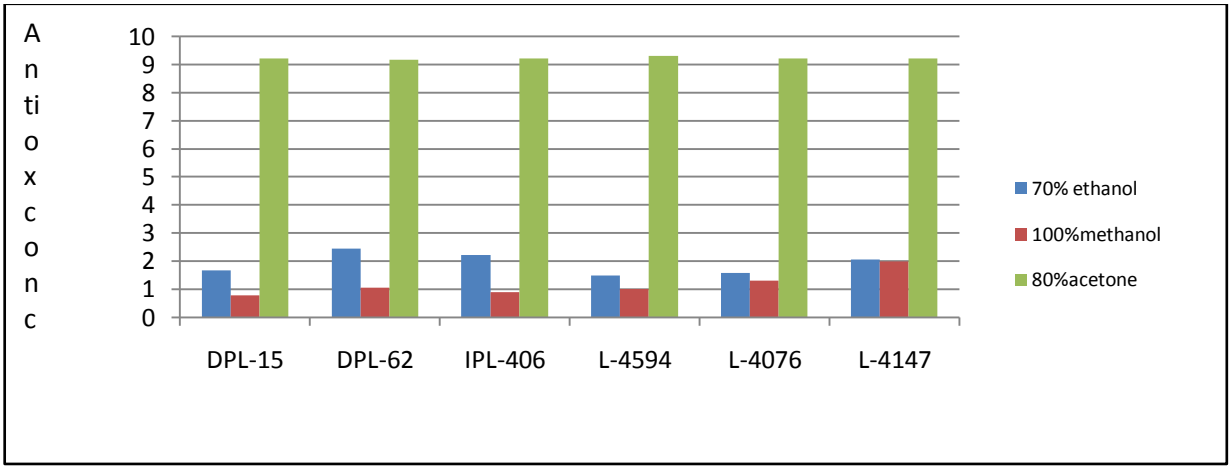


Fig 3: FRAP (mMol Fe^{2+}/g) of different lentil cultivars as affected by different solvents.



CONCLUSIONS

The present study indicates that Indian cultivars of lentils exhibit high phytochemical content and antioxidant activity and could be source of important dietary bioactive compounds with potential health benefits. Further research is needed to elucidate the composition of the seed extract for identification and level of bioactive compounds. The impact of food processing methods as well as physiological processes like digestion on the stability of these phytochemicals and their antioxidant activity needs to be established in order to use lentils as natural therapeutic food supplement.

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