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Antioxidant Activity of Pterocarpan (*Phaseolus vulgaris*) A Priliminary Assesment of Crude Extracts

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ABSTRACT

The antioxidant activity of 80% ethanol and 70% acetone extracts of Pterocarpan (*Phaseolus vulgaris*) form dry seedsand podwere evaluated to determine their feasibility as natural antioxidants. The results showed that crude extracts were rich in total Phenolic compounds and had clear antioxidant activities. The free radical-scavenging capacity of the Pterocarpanextracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The extracts obtained from dry seeds EC50 values of 0.14 g dm/g DPPH (acetone extract) and 0.20 g dm/g DPPH (ethanol extract) while the extracts obtained from pod present EC50 values of 0.26 g dm/g DPPH (acetone extract) and 0.37 g dm/g DPPH (ethanol extract). There are significant correlations between the total content of polyphenols and the antioxidant activity (R2 = 0.9352) and between the flavanols content and the antioxidant activity (R2 = 0.9404) of the Pterocarpanextracts obtained

Keywords: Antioxidant Activity, Seed Extracts, Polyphenols, Pterocarpan, Radical Scavenging Activity, DPPH

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INTRODUCTION

The importance of leafy vegetables in nutrition and health cannot be overemphasized. According to Lu and Foo [8] a diet rich in vegetables is recommended along with fruits and whole grains since such a diet has an inverse association with the risk of chronic diseases. In recent times the medicinal value of plant source foods has assumed a more important dimension owing to the discovery that their extracts contain not only micronutrients but also a diverse array of secondary metabolites with antioxidant potential. Vegetables are known to contain antioxidants necessary in neutralizing free radicals which are known human chemical hazards. Free radicals are generated under a number of conditions such as drinking alcohol, smoking and exercise [1]. They are, however, often produced in normal cellular metabolism due to oxidation of biomolecules for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing [1] are protected against free radical damage by oxidative enzymes such as Superoxide Dismutase (SOD) and Catalase (CAT), or chemical compounds mostly found in plant foods such as "-tocopherol, ascorbic acid, carotenoids, polyphenols and glutathione [11]. Many plants have been identified as good sources of natural antioxidants such as tocopherols, vitamin C, carotenoids and polyphenols which are responsible for maintaining good health and protect against coronary heart diseases and cancer [2, 5-7]. Hence, free radicals accumulate when the mechanism of antioxidant protection becomes unbalanced. However, available evidence indicates that reparative processes do not fully eliminate free radical-induced damage of biological macromolecules. Thus, a more effective way is the prevention of oxidant induced damage by reducing the levels of reactive chemical species with unpaired electrons (free radicals) to the barest minimum and reinforcing natural antioxidant action [9]. For this reason natural antioxidant-containing foods, such as vegetables and spices, are being extensively studied for their capacity to protect cells from damage brought on by oxidative stress. The French bean offers a variety of vegetables as food resources but lot of work has been done to ROS components in these vegetables in order to understand their fullest nutritional and health benefits. It is against this background that this study was conducted to evaluate the seed and pods confer antioxidant properties of *French bean (Phaseolus vulgaris)* with the aim of determining the changes in concentration and suggesting the both the parts has antioxidant properties.

MATERIALS AND METHODS

Materials and Chemicals

French bean (*Phaseolus vulgaris*) S-9 *varieties*, widely grown as the major vegetable cropin India, and other parts of the country. The mature seed and fresh pod used in this study. Samples were dried, ground and stored at −20°C until used.

The following standards and reagents were used: Stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) was obtained from SigmaAldrich, Germany. Folin-Ciocalteau reagent

was purchased from Merck, Germany. (+)- Catechin and DL-α-tocopherol from Sigma, Spain, and Gallic acid from Aldrich, Germany. All reagents used were of analytical grade. Dry matter was determined by drying the samples to a constant weight at 105°C.

Preparation of Extracts

Pterocarpanextracts from French bean were prepared using two solvents ethanol: water (80/20, v/v), in some cases, or acetone: water (70/30, v/v), in the others. Pterocarpanwas extracted twice with the selected solvent at room temperature for 1 hour with continuous stirring in each case. The ratio of solvent used was 10mL per gram of sample. After centrifugation, supernatants were combined, concentrated at 40°C and lyophilised. The solid dry extracts were subjected to successive analyses.

Determination of Total Polyphenols and Flavanols

Total polyphenols were spectro-photometrically determined in the solid dry extracts by reading the absorbance at 765 nm, using gallic acid as a standard and expressing the results as gallic acid equivalents (GAE). Flavanols were quantified in the solid dry extracts by the vanillin assay using (+)-catechin as standard and expressing the results as (+)-catechin equivalents (CAE). [10,13]

Determination of Antioxidant Activity

The antioxidant activity of the Pterocarpansolid extracts was measured in terms of radical scavenging ability, according to the DPPH method reported by (Brand-Williams *et al.* modified by Sánchez-Moreno *et al* [4, 12]. The EC50 parameter, which reflects 50% depletion of DPPH free- radical, was expressed as grams of dry extract per gram of DPPH. This characteristic parameter is called efficient concentration (EC50) or oxidation index, and the lower the value, the higher the antioxidant activity of the examined product. For rational reasons of clarity, the antiradical activity AAR was determined as the inverse value of the efficient concentration EC50, representing a comparable term for the effectiveness of antioxidant and radical scavenging capacity: AAR = 1/EC50. The larger the AAR, the more efficient is the antioxidant. For comparison purposes, the efficient concentrations of vitamin C and vitamin E were also evaluated.

Statistical Analysis

The determinations of total polyphenols and flavanol contents, and the antioxidant activity were carried out at least in quadruplicate. Results are presented as the mean value with the standard deviation (SD). Correlation between total polyphenols and flavanol contents and anti- oxidant activity was verified by the determination coefficient R2 of the linear regression.

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RESULTS AND DISCUSSION

Yield, Total Polyphenolic and Flavanol Content of Solid Extracts

Table 1 shows the yield obtained for aPterocarpan (Phaseolus vulgaris). The values were close to 30% of seed dry weight (27.3% - 32.1%). Concentration of total polyphenols and flavanols obtained for the Seed extracts are shown in **Table 1** and are referred to as g to 100 g dry matter (dm). The pterocarpansolid extracts obtained from an 80% ethanol extraction process are RS-ETOH (pod) and WS-ETOH (from seeds) and those corresponding to 70% acetone extraction process are RS- ACET (pod) and WS-ACET (seed). The high poly-phenolic content for all the extracts can be observed in **Table 1**. The quantities of total polyphenols present in the podextracts were higher than those of the seed extracts, independent of the solvent used. Concentrations of total polyphenols in the RS-ETOH and RS-ACET extracts were equal to 29.4 and 38.4 (g GAE/100 g dm), respectively; whereas the quantities of polyphenols contained in the related extracts obtained from seed, WS-ETOH and WS-ACET, were 17.2 and 22.9 (g GAE/ 100 g dm), respectively. This fact is in accordance with the higher polyphenolic content of the pod S-9 in opposite to the seed variety S-9. Moreover, **Table 1** illustrates that the acetone extracts obtained from either seed were richer in polyphenols than that the ethanol extracts. In fact, for the pterocarpanextracts corresponding to the pod, RS-ACET was richer than RS-ETOH (38.4 to 29.4 g GAE/100 g dm, respectively), and analogously, for pterocarpanextracts corresponding to the seed, WS-ACET was richer than WS-ETOH (22.9 to 17.2 g GAE/100 g dm, respectively).

Therefore, the results show the high polyphenolic content for all the pterocarpansolid extracts obtained in this work that are between 17.2% and 38.4% of dm (**Table 1**). Additionally, **Table 1** gives the flavanol content of the extracts. Similar in pattern to the results shown above for the polyphenols, the flavanol content of the pod extracts, RS-ETOH and RS-ACET, were higher than that in the seed extract, WS-ETOH and WS-ACET (21.7 and 27 g CAE/100 g dm compared to 12.6 and 16.2 g CAE/100 g dm, respectively). Again, the acetone extract obtained from either pod or seeds of S-9 varieties was richer in flavanols than that the ethanol extract. Notable is the high flavanol content for all the pterocarpanextracts obtained. **Table 1** clearly shows that flavanols accounted for approximately 70% of the polyphenolic content of seed and pod extracts. Summarizing, the best pterocarpanextract for either total polyphenol or flavanols contents was that obtained from a pod, using 70% acetone as solvent.

Antioxidant Activity

Table 2 shows the antioxidant activity of pod and seed extracts expressed as effective concentration, EC50, antiradical activity, AAR and antioxidant activity equivalents of vitamin C and vitamin E. All Pterocarpanextracts obtained presented a high antioxidant activity, especially the pod extracts, RS-ACET and RS-ETOH, which have an EC50 = 0.14 and 0.20 g dm/ g DPPH, respectively, representing an antiradical activity AAR of 7.14 and 5 g DPPH/ g dm, respectively. These high antioxidant activities correspond to 615 and 430 vitamin C mg-equivalent/g dm,

respectively, and 1630 and 1140 vitamin E mg-equivalent/g dm, respectively. Note that both extracts had an antioxidant activity higher than that vitamin E. On the other hand, the extracts obtained from the seed extract, WS-ACET and WS-ETOH, had an EC50 = 0.26 and 0.37 g dm/g DPPH, respectively, representing an antiradical activity AAR of 3.85 and 2.70 g DPPH/g dm, respectively. They corresponded to 331 and 233 vitamin C mg-equivalent/g dm, respectively, and 878 and 616 vitamin E mg-equivalent/g dm, respectively. The Pterocarpan extract with a higher antioxidant activity was that obtained from pod using 70% acetone as solvent. Is should be noted that this follows that same trend as in the cases of total polyphenols and flavanols previously mentioned. In fact, antioxidant activity was closely correlated with the total content of polyphenols and flavanols. So, by linear regression analysis, there is a significant correlation between the total content of polyphenols and the antioxidant activity of the Pterocarpanextracts studied. The linear equation resulting was: Polyphenols content = 48.801 - 89.962 EC50 (determination coefficient, R2 = 0.9352) (negative slope due to the lower the EC50 value, the higher the antioxidant activity). This fact was described by several authors in selected fruits, vegetables and grain products**,** tea infusion, *Rubus*species and vine grapes and leaves [3, 14]. These authors found a significant correlation between the total content of poly- phenols and the antioxidant power. Moreover, there is a significant correlation between the flavanols content and the antioxidant activity of thePterocarpanextracts studied and the linear equation re-sulting was: Flavanols content = 34.550 - 62.588 EC50 (determination coefficient, R2 = 0.9404). The use of natural polyphenols from grape stem as food additives can be advantageous. It is well known that the maximum lawful levels for synthetic food antioxidants are established from different toxicological parameters that need not be applicable to naturally occur- ring compounds. Therefore, the polyphenols from natural extracts could be used at higher levels than the synthetic phenols, thereby increasing their antioxidant effective- ness. Also, the anti-oxidative characteristics of these natural compounds can represent an interesting feature for their application to prevent deterioration of pharmaceuticals and cosmetics.

Table 1: Yield, total polyphenols and flavanols of Pterocarpan Extracts

Solid Extracts	Yield (% dm)	Polyphenols (g GAE/100 g dm)	Flavanols (g CAE/100 g dm)
RS-ETOH	29.5 ± 0.6	29.4 ± 0.4	21.7 ± 0.3
WS-ETOH	27.3 ± 0.5	17.2 ± 0.2	12.6 ± 0.2
RS-ACET	30.1 ± 0.6	38.4 ± 0.5	27.0 ± 0.4
WS-ACET	32.1 ± 0.6	22.9 ± 0.3	16.2 ± 0.2

Table 2: Effective concentration EC50, antiradical activity, AAR and antioxidant activity equivalents of vitamins C and E of Pterocarpan extracts.

Solid Extract	EC50 (g dm/g DPPH)	AAR (1/EC50)	AA-Vit C eq (ascorbic acid) (vit. C mg-eq/g dm)	AA-Vit Eeq(DL-α-tocopherol) (vit. E mg-eq/g dm)
RS-ETOH	0.20 ± 0.02	5.00 ± 0.50	430	1140
WS-ETOH	0.37 ± 0.04	2.70 ± 0.29	233	616
RS-ACET	0.14 ± 0.01	7.14 ± 0.71	615	1630
WS-ACET	0.26 ± 0.03	3.85 ± 0.44	331	878

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CONCLUSIONS

The high contents in total polyphenols as well as in flavanols together with the excellent antioxidant properties of all the Pterocarpan extracts obtained confer a wide range of applications of these products in food, cosmetic and pharmacological industries. Taking into account that grapes are used on the order of millions of tons in wine producing countries, the results for these four solid extracts obtained from Pterocarpan indicate an abundant and valuable source of natural antioxidant sources in which their utilization could also provide at least a partial solution for the vegetable consumption.

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