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PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF GREEN GRAM WHOLE (PHASEOLOUS AUREUS ROXB)

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ABSTRACT

Green gram whole (Phaseolous Aureus Roxb), besides being rich in conventional nutrients i.e. carbohydrates, protein, vitamins and minerals, now-a-days extolled as an excellent functional food attributed to the presence of phenols, flavonoids and other bioactive compounds. The objective of this study was to evaluate the phytochemical profile and antioxidant activity of methanolic-acetone extracts obtained from Green gram whole (Phaseolous Aureus Roxb). The antioxidant activity was assessed by DPPH (2,2-Diphenyl-1-picrylhydracyl) radical scavenging assay, ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay and FRAP (Ferric reducing antioxidant power) assay. The results showed this legume to be a good antioxidant food source as DPPH, ABTS and FRAP assay values stood at 429.53±18.8mg Trolox equivalent/100g, 194.73±9.69mg Trolox equivalent/100g and 1240.96±19.80mg ferrous sulphate/100g respectively. Of the phytochemical profile, total phenols and flavonoids were found to be 400.91±26.45mg gallic acid equivalent/100g and 144.96±5.15mg catechin equivalent/100g respectively. Correlation analyses exhibited poor correlation between total phenols and flavonoids, antioxidant activity assays while statistically significant correlation between flavonoids and antioxidant activity assays. Overall, green gram whole validates itself to be a potential functional food ingredient as an antidote to chronic, degenerative diseases.

KEYWORDS: whole green gram, phytochemicals, phenolics, antioxidant activity.

ABBREVIATIONS

DPPH: 2,2-Diphenyl-1-picrylhydracyl, ABTS: 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) , FRAP: Ferric reducing antioxidant power, TPC: Total phenolic content, TFC: Total flavonoid content, TE: Trolox equivalent, GAE: Gallic acid equivalent, CE: Catechin equivalent, TROLOX: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

I. INTRODUCTION

Food is essential for sustainence of life and wellbeing. Besides meeting basic nutritional needs of energy-yielding, body-building and protection, the recently purported terms like "functional foods", "nutraceuticals", "foods for specific health use" or "specific health promoting foods" suggest that foods may have a beneficial action or certain functions in the organisms that goes beyond the nutritional effects.

Robust research evidence highlights phytochemicals to have gained unprecedented recognition as "Life Span Essentials". Phytochemicals are non-nutrient, bioactive chemical compounds that are produced by plants; and within biological systems, they act as prolific dietary antioxidant and render anticarcinogenic, anti-inflammatory, cardioprotective and immunity boosting effects. About 1000 phytochemicals are known till date. Foods i.e. fruits, vegetables, legumes, beans manifest an excellent dietary vehicle to provide wide range of phytochemicals(Liu Rh, 2004).

Green gram, whole (*Phaseollus aureus Roxb*) is extolled as an excellent source of nutrient- and non-nutrient antioxidants, besides being rich in macronutrients with complete essential amino acids profile and abundant in micronutrients i.e. potassium, magnesium, calcium, phosphorus, iron, carotene, thiamine, niacin, riboflavin and folic acid (Kavya et al, 2014). Nutrient antioxidants entail tocopherols (having their predominance as $\gamma > \alpha > \delta$-

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tocopherols) and ascorbic acid (Kim et al, 2014) while non-nutrient antioxidant scaffold include phenolic components like Hydroxybenzoic acids (eg. gallic, protocatechuic, gentisic, *p*-hydroxybenzoic, *ß*-resorcylic, vanillic, syringic, veratric and salicylic acids); Hydroxycinnamic acids (eg. chlorogenic, quininc, *p*-coumaric, ferulic, *m*-coumaric, *t*-cinnamic and caffeic acids); Flavones (vitexin, isovitexin); Flavanones (eg. hesperidin, rutinose, hesperetin, naringin and naringenin); Flavonols (eg, quercetin, kaempferol, myricetin, rutin), Flavanol (eg. Catechin), Isoflavones (eg. formononetin, biochanin A), resveratrol, pyrogallol, vanillin, homogentisic acid (Kim et al, 2013).

II. MATERIALS AND METHODS

Sample procurement: The pulse variety IPM 02-3 was procured from Krishi Vigyan Center (ICAR), Banasthali University. Samples were washed, dried in shade and then hand sorted to remove wrinkled seeds and foreign material and thereafter, stored in air tight container for further use.

Chemicals: Folin-ciocalteau reagent, sodium carbonate, gallic acid, aluminium chloride hexahydrate (AlCl3.6H2O), NaNO2, sodium hydroxide (NaOH), catechin, Ethanol, DPPH, ABTS, TROLOX, potassium persulphate, sodium acetate trihydrate, glacial acetic acid, 2,4,6-tripyridyl-S-triazine (TPTZ), Conc. hydrochloric acid (HCl), ferric chloride hexahydrate (FeCl3.6H2O), Ferrous sulphate heptahydrate (FeSO4.7H2O), deionized water were obtained from Sigma Chemicals and Merck.

Sample preparation: the sample was pulverized using home grinder and was used immediately for phenolic extraction.

Sample extraction: Pulverized samples (0.25g) were placed in test tubes with 10ml of methanol/water (50:50, v/v). The pH was adjusted to 2 using 2M HCl. The tubes were thoroughly shaken, using orbital shaker, at room temperature for 1 hr, and then centrigued at 2500 g for 10 mins. Supernatants were collected in clean dry test tubes. Then the residues were extracted again with 10 ml of an acetone/water mixture (70:30, v/v). The methanol and acetone extracts were combined and subsequently used for various assays. Extracts produced in duplicate. In case of non-usage of extracts on the same day, they were stored at 4°C and used within a week for all analysis.

Phytochemical analysis

1) Determination of Total phenolic content (TPC)

Principle: It is based on the single electron transfer (SET) in alkaline medium (7% $NaCO_3$) from phenolic compound to molybdenum, forming blue complex which is measured spectrophotometrically at 750-765nm.

Procedure: TPC was determined according to Xu and Chang, 2007 using Folin-Ciocalteu assay and having gallic acid as standard. The mixture of sample solution (0.1 ml), deionized water (6 ml), FCR solution (0.5 ml), 7% $NaCO_3$ (1.5 ml) was vortexed for 1 min and incubated for 8 min at room temperature. Then a dose of 1.9 ml of deionized water was added. The mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765 nm against blank.

Calculations: TPC was expressed as gallic acid equivalent (mg GAE/100g sample) through the calibration curve of gallic acid. Linearity range of the calibration curve was 100 to 1000 µg/ml.

2) Determination of Total flavonoid content (TFC)

Principle: Aluminum chloride forms acid complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonois. In addition, aluminum chloride forms acid labile complexes with orthodihydroxyl groups in the A or B ring of flavonoids.

Procedure: TFC was determined according to Xu and Chang, 2007. 0.5 ml of sample was mixed with 2.5 ml of deionized water in a test tube followed by adding 0.15 ml of 5% sodium nitrite ($NaNO_2$) solution. After 6 minutes, 0.3 ml of 10% aluminum chloride hexahydrate ($AlCl_3.6H_2O$) solution was added and allowed to stand for another 5 minutes before adding 0.1 ml of 1 M NaOH. The mixture was brought to 5 ml with the addition of 0.55 ml of deionized water and mixed well. The absorbance was measured immediately against the blank (the same mixture without the sample) at 510 nm using UV-spectrophotometer.

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Calculations: TFC was expressed as catechin equivalents (mg CE/100g sample). Linearity range of catechin calibration curve was 62.5-375 µg/ml.

Antioxidant activity analysis

1) DPPH radical scavenging activity

Principle: It is based on antioxidant-catalyzed reduction of purple-colored DPPH radical to its yellow-colored non-radical form which is measured spectrophotometrically at 517nm.

Procedure: This assay was conducted according toXu and Chang, 2007. A doseof 0.2 ml of tested legume extract was added to 7.6 ml ethanol solution of DPPH radical (final concentration was 0.1 mM). The mixture was shaken vigorously for 1 min by vortexing and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance was measured at 517 nm against ethanol blank.

Calculations: DPPH scavenging activity was expressed as trolox equivalents (mg TE/100g sample). Linearity range of trolox calibration curve was **0**.05 µM to 0.4 mM trolox.

2) ABTS radical scavenging activity

Principle: It is based on antioxidant inhibition of the absorbance of blue-green coloured ABTS radical, generated via persulfate-induced ABTS oxidation, measured spectrophotometrically at 734nm.

Procedure: This assay was conducted according toThaipong et al., 2006. Thestock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml $ABTS^{\bullet+}$ solution with 30 ml methanol to obtain an absorbance of 1.1 ±0.02 units at 734 nm using the spectrophotometer. In sample (0.3 ml), prepared $ABTS^{\bullet+}$ solution (5.7 ml) was added and kept it for 2 hrs in a dark condition. Then the absorbance was taken at 734 nm using spectrophotometer.

Calculations: ABTS scavenging activity was expressed as trolox equivalents (mg TE/100g sample). Linaerity range of trolox calibration curve was 0**.**1-0**.**7 mM trolox.

3) FRAP assay

Principle: It is based on antioxidant-catalyzed reduction of ferric-TPTZ complex (colorless) to ferrous form (intensely blue coloured) which is measured spectrophotometrically at 593nm

Procedure: This assay was conducted according to Xu and Chang, 2007. The working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10mM TPTZ (2,4,6-Tripyridyl-s-triazine) in 40mM HCl and with 1 volume of 20mM FeCl3 ×6H2O (ferric chloride hexahydrate). Prepared working FRAP reagent was warmed to 37◦C. In 6 ml FRAP reagent, 0.2 ml of sample and 0.6 ml of deionized water were added and the absorbance was taken at 593 nm against reagent blank after 4min.

Calculations: FRAP value was expressed as Fe^{2+} (Iron (II) sulfate heptahydrate or ferrous sulfate) equivalent (mg FRAP/100g sample). Linearity range of the calibration curve was 0.1-0.9mM.

III. RESULTS AND DISCUSSION

Plant foods are ubiquitous and excellent source of phytochemicals known to act as potent antioxidants in biological systems. The present study was endeavoured to determine the phytochemical profile (total phenols and flavonoids) and associated antioxidant activity of green gram whole. The antioxidant activities were expressed as DPPH radical scavenging activity, ABTS radical scavenging activity and FRAP assay. Aqueous extracts (methanolic acetone extracts) of green gram whole subjected to these tests showed significant antioxidant activity. The results of its phytochemical profile and antioxidant activity (DPPH, ABTS and FRAP) are arranged in Table 1 and 2 respectively. Its TPC and TFC values are in agreement with the study of Hithamani and Srinivasan 2014, values being 403mg GAE/100g and 156mg catechin/100g respectively. Its mean DPPH, ABTS and FRAP values corroborates with the studies cited by Baojun and Chang 2012, Marathe et al 2011 and Petchiammal and Hopper 2014 respectively. This suggests that the aqueous extract of green gram whole contain phytochemicals in considerable amounts and have potential to donate hydrogen atom to

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unstable free radicals and protect us from diseases associated with free radical damage. Furthermore, the phenolic constituents documented to be present in this maverick pulse are recently documented to provide disease protection through various molecular mechanisms, even at low blood phenolic concentrations.

Table 1: Phytochemical profile of aqueous-organic extracts of green gram, whole

Sample	Botanical name	TPC (mg GAE/100g)	TFC (mg CE/100g)
Green gram, whole	*Phaseolous aureus* Roxb	400.91±26.45	144.96±5.15

[Values are mean ± SD; N = 3]

Table 2: Antioxidant activity of aqueous-organic extracts of green gram, whole

Sample	Botanical name	DPPH (mg TE/100g)	ABTS (mg TE/100g)	FRAP (mg $FeSO_4$/100g)
Green gram, whole	*Phaseolous aureus* Roxb	429.53±18.81	194.73±9.69	1240.96±19.80

[Values are mean ± SD; N = 3]

The results from correlation analyses between phenolic contents and antioxidant activities in the aqueous extract of green gram, whole are given in table 3. Correlation coefficient between TPC and antioxidant activities as determined by three methods i.e. DPPH, ABTS and FRAP, and between TPC and TFC show lack of correlation which could be due to different responses of different phenolic compounds in different assay systems, marked molecular antioxidant responses of phenolic compounds based on their chemical structure (Marja et al,1999), and employing Folin-Ciocalteu reagent, which other than phenolic compounds, is known to react with sugars and ascorbic acid in plant extracts (Matthaus, 2002). However, correlations between TFC and three antioxidant activity assays, and correlation among different antioxidant assays was found to be significant. Statistical significant correlations were observed between TFC-ABTS (.995, <0.05), DPPH-FRAP (.998, <0.05) and ABTS-FRAP (1.000, <0.05).

Table 3: Correlations between phenolic contents and antioxidant activities of aqueous-organic extracts from Green gram, whole

	TPC	TFC	DPPH	ABTS	FRAP
TPC	-	-	-	-	-
TFC	.243	-	-	-	-
DPPH	.397	.987	-	-	-
ABTS	.312	.997*	.996	-	-
FRAP	.338	.995	.998*	1.000*	-

*Correlation is significant at 0.05 level (2-tailed)

IV. CONCLUSION

Green gram, whole (*Phaseolous Aureus* Roxb) is a maverick legume. Besides being rich in energy and having promising streaks in basic nutrition i.e. complete essential amino acids profile, high is resistant starch, excellent food source of vitamins and minerals, this legume is extolled as excellent functional food worldwide attributed to being exquisite source of dietary fiber, nutrient- and non nutrient antioxidants and other bioactive components. This study subjected to analyze the phytochemical profile and antioxidant activity of green gram, whole validate it to be a profound antioxidant food and nutraceutical substance having potential ability to mitigate the risk of chronic and degenerative diseases..

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