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Article

Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds

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The effect of solvent polarity on extraction yield and antioxidant properties of phytochemical compounds in bean seeds was studied. Seed flour of three varieties of bean was extracted in a series of organic solvents with increasing polarity (*n*-hexane, petroleum ether, chloroform, ethyl acetate, ethanol, acetone and water). Preliminary screening of phytochemicals showed the presence of tannins, flavonoids, cardiac glycosides, anthocyanins, terpenoids, carotenoids, ascorbic acid and reducing compounds in all extracts. One way analysis of variance (ANOVA) of results showed that extraction yield, phytochemical content and antioxidant properties were significantly influenced ($p<0.05$) by the polarity of extracting solvents. The regression analysis of data showed polarity-dependent second order polynomial variations in the extraction yield, phytochemical contents, antioxidant activity, reducing properties and free radical scavenging activity of each variety. Extraction in highly polar solvents resulted in high extract yield but low phenolic and flavonoid content as compared to non-polar ones. The polarity-dependent increase in total antioxidant activity and reducing properties indicates the extraction of strong antioxidant compounds in polar solvents. The study suggests the use of a combination of polar and nonpolar solvents to increase the extraction efficiency of phytochemicals with good antioxidant quality from the bean and other legume seeds.

Keywords: Beans seeds/antioxidant properties. *Phaseolus vulgaris*/phytochemical composition. Regression analysis. Solvent polarity.

INTRODUCTION

Phytochemicals are non-nutrient bioactive compounds found in fruits, vegetables, cereals, legumes and most of the medicinal plants. These compounds possess antioxidant properties and reduce the risk of oxidative damages imposed by free radicals produced during the normal metabolism (Guevara-González, 2006; Brewer, 2011; Coles, 2013). The human body has developed efficient mechanisms in order to neutralize the adverse effects of oxidative damage either by blocking the production of free radicals or by enhancing the production of endogenous antioxidants (Masella *et al*., 2005). Antioxidant compounds also help to delay and inhibit the lipid oxidation process to minimize the rancidity, retard the formation of toxic products, help to maintain the nutritional quality and increase the shelf life of food materials (Brewer, 2011; Coles, 2013).

The extraction and purification of phytochemical and antioxidant substances from the plant material are generally affected by various factors including time, temperature, solvent concentration and solvent polarity. Depending on chemical nature, various phytochemicals are extracted in solvents of different polarity as no single solvent may be reliable to extract all the phytochemical and antioxidant compounds present in the plant material (Lapornik, Prosek, Wondra, 2005; Iloki-Assanga *et al*., 2015). Serial exhaustive extraction method involves the successive extraction with solvents of increasing polarity from non-polar (*n*-hexane) to more polar solvent (water) to ensure the extraction of a wide range of compounds with different polarity (Das, Tiwari, Shrivastava, 2010; Bimakr *et al*., 2011; Abdel-Aal, Haroon, Mofeed, 2015). Studies have reported that solvent polarity significantly affects the extract yield and antioxidant activity of phenolic compounds in plant material (Ghasemzadeh,

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Jaafar, Rahmat, 2011; Ghasemzadeh *et al.*, 2015; Barchan *et al*., 2014).

Beans botanically known as *Phaseolus vulgaris* have been used as foodstuff and fodder for cattle throughout the world. It has been reported that commonly used species of the bean are the good source of proteins, carbohydrates, fiber and minerals along with significant amount of phytochemical and antioxidant compounds. Bean seeds are medicinally important due to their antioxidant, anticancer, antimicrobial, antiobesity, cardioprotective, hepatoprotective and antiproliferative activities (Guevara-González, 2006; Zhu, Jiang, Thompson, 2012; Guajardo-Flores, Serna-Saldívar, Gutiérrez-Uribe, 2013; Ortega *et al*., 2013; Zou, Chang, 2014). In continuity of previous findings, we planned to investigate the effect of solvent polarity on extraction efficiency of phytochemical compounds and antioxidant activity of three varieties of bean (white beans, red kidney beans and small common beans) by consecutive extraction in a series of seven solvents with increasing polarity order (*n*-hexane, petroleum ether, chloroform, ethyl acetate, ethanol, acetone and water). The results may provide significant data on the suitability of solvents for the extraction of phytochemicals and antioxidants from plant materials particularly beans.

MATERIAL AND METHODS

Sampling

The dried seeds of three varieties of beans (white beans, red kidney beans and small common beans) were purchased from the local market and transported to the laboratory. The mature and healthy seeds were separated manually, cleaned from dust, ground to a fine powder using pestle and mortar and sieved through an 80 mesh sieve to obtain the sample of fine particle size (<50µm). The flour obtained was dried under shade at 35±5ºC to minimize the moisture content (<2%), packed in air tight black coated glass jars and stored at sterile laboratory conditions until further analysis.

Preparation of extracts

The extracts were prepared by consecutive extraction method using a series of organic solvents with increasing polarity order in terms of their dipole moments (hexane: 0.0, petroleum ether: 0.1, chloroform: 4.1, ethyl acetate: 4.4, ethanol: 4.9, acetone: 5.1 and water: 9.2 D). The flow sheet for entire extraction procedure is given in Figure 1. The seed flour (20 g) was extracted in *n*-hexane (100 mL) for 24 h at room temperature (25±5 ºC) using mechanical shaker (KS/HS 501 Digital Orbital And Reciprocal Shaker, Thomas Scientific Inc. USA). The extract was evaporated to dryness, weighed and the total extractable components (TEC) were calculated using Eq. 1.

$$TEC(\%) = \frac{Weight\ of\ extract}{Weight\ of\ sample} \times 100 \qquad (1)$$

The residue obtained after extraction in hexane was further extracted consecutively with petroleum ether, chloroform, ethyl acetate, ethanol, acetone and water. The residue obtained in previous extraction step was extracted in next solvent and the extracts obtained in each extraction step were collected, dried and weighed for calculation of TEC as shown in flow sheet. The dried extracts were then dissolved in the respective solvent (10 mg/100 mL) and used for phytochemical and antioxidant analysis.

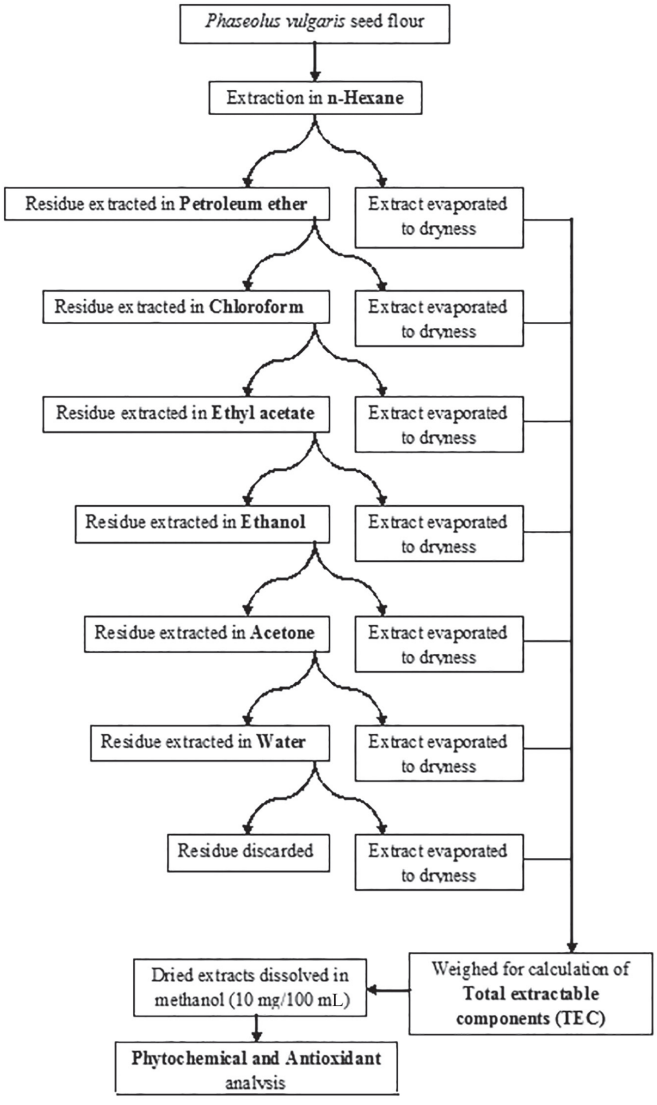

FIGURE 1 - Experimental scheme for extraction of phytochemicals.

Preliminary screening of phytochemicals

Preliminary screening of phytochemicals including tannins, saponins, flavonoids, cardiac glycosides, anthocyanins, terpenoids, phlobatannins, free anthraquinones, bound anthraquinones, carotenoids and reducing compounds present in bean seeds was done by methods described earlier (Sofowora, 1982; Trease, Evans, 1989; Harborne, 1998).

Phytochemical and antioxidant analysis

Total phenolic content (TPC) was determined by previously described Folin-Ciocalteu's method (Shad *et al*., 2012). TPC were calculated as gallic acid equivalent g/100g dry wt. using the regression equation (Eq. 2) obtained from the standard curve of gallic acid (R^2=*0.987*).

$$TPC\left(\frac{g}{100\,g\ dry\ wt.}\right) = Abs.at\ 720\ nm / 2.45 \quad (2)$$

Total flavonoid content (TFC) was determined by reported method (Soares *et al*., 2003) with few modifications. TFC was calculated as catechin equivalent g/100 g dry wt. using following regression equation (Eq. 3) obtained from the standard curve of catechin anhydrate (R^2=*0.996*).

$$TFC\left(\frac{g}{100\,g\ dry\ wt.}\right) = Abs.at\ 500\ nm / 4.64 \quad (3)$$

Trolox equivalent total antioxidant activity (TAOA) of extracts was determined by previously described phosphomolybdenum assay (Prieto, Pineda, Aguilar, 1999). TAOA was calculated as g/100 g dry wt. using following regression equation (Eq. 4) obtained from the calibration curve of Trolox (R^2=*0.9772*):

$$TETAOA\left(\frac{g}{100\,g\ dry\ wt.}\right) = Abs.at\ 695\ nm / 1.29 \quad (4)$$

Reducing potential of extracts was determined in terms of iron reducing capacity (IRC) and linoleic acid reducing capacity (LARC) by the methods reported earlier (Osawa, Namiki, 1981; Oyaizu, 1986).

Free radical scavenging capacities of extracts were determined in terms of hydroxyl radical scavenging capacity (HRSC) and DPPH [2,2-diphenyl-1-picryl hydrazyl] radical scavenging capacity (DPPH RSC) by using procedures reported in the literature (Smirnoff, Cumbes, 1989; Sánchez-Moreno, Larrauri, Saura-Calixto, 1998).

Statistical analysis

All the procedures for extraction, phytochemical analysis, and antioxidant studies were repeated in triplicate. The results were expressed as means± standard deviation of three parallel replicates. The means were separated by one-way analysis of variance (ANOVA) at confidence level $p \leq 0.05$ by applying Tukey's multiple range tests using statistical software SPSS version 19.0. The data were analyzed by regression model to study the polarity dependent variation in phytochemical composition and antioxidant profile of bean seed extracts.

RESULTS AND DISCUSSION

Phytochemical screening of bean seeds extracts in various solvents confirmed the presence of tannins, flavonoids, cardiac glycosides, anthocyanins, terpenoids, carotenoids, reducing compounds and ascorbic acid in each extract of each variety (Table I). However, the presence of saponins, phlobatanins and anthraquinones were not confirmed in any of the extracts. The results were in partial agreement with those reported earlier in bean seeds (Atchibri *et al*., 2014; Pradeepkumar *et al*., 2015).

The effect of solvent polarity on extraction efficiency of phytochemical and antioxidant compounds of three varieties of bean was studied by consecutive extraction in a series of solvents with increasing polarity (n-hexane, petroleum ether, chloroform, ethyl acetate, ethyl acetate, ethanol, acetone and water). The extract yield was calculated as total extractable components (TEC) which ranged from 0.24±0.11-7.23±0.47 g/100 g dry wt. (Figure 2A). Total phenolic content (TPC) and total flavonoids content (TFC) of the extracts ranged from 10±4-101±10 and 8±2.4-70±9.05 mg/100 g dry wt. respectively while Trolox equivalent total antioxidant activity (TAOA) ranged from 0.15±0.05-2.53±0.23 g/100 g dry wt. (Figure 2B-D). Reducing potential of extracts was estimated in terms of iron reducing capacity (IRC) and linoleic acid reduction capacity (LARC) which ranged from 0.18±0.08-0.75±0.65 (Abs. at λ=700 nm) and 18.50±2.61-47±3.31% respectively (Figure 3A, B). Free radical scavenging capacities of extracts were determined as hydroxyl radical scavenging capacity (HRSC) and DPPH radical scavenging capacity (DPPH RSC) which were found to be in the range of 32±2.92-93±4.40 and 18±2.10-43±3.12% respectively (Figure 3C, D).

One way analysis of variance (ANOVA) of the results showed that extraction yield, phytochemical content and antioxidant properties of bean seeds were found to be significantly influenced (p<0.05) by increasing

the polarity of extracting solvents except for TFC and IRC of white beans. The regression analysis of data showed a polarity dependent polynomial variation in all of the studied parameters of each bean variety. The generalized polynomial regression equations obtained by suggested model, regression coefficients and significance values for studied parameters are given in Table II.

A significant positive polynomial response of TEC

TABLE I - Phytochemical screening of bean seeds extracts in various solvents

Screening tests	White beans							Red kidney beans							Small common beans						
	HE*	PeE	ChE	EaE	EE	AE	WE	HE	PeE	ChE	EaE	EE	AE	WE	HE	PeE	ChE	EaE	EE	AE	WE
Tannins	+**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carotenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reducing Compounds	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascorbic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* HE: Hexane extract (0), PeE: Petroleum ether extract (0.1), ChE: Chloroform extract (4.1), EaE: Ethyl acetate extract (4.4), EE: Ethanol extract (4.9), AE: Acetone extract (5.1), WE: Water extract (9.2). Values in parenthesis indicate the polarity of respective solvent in terms of its dipole moment (D). **positive (+) and negative (-) signs indicate the presence and absence of phytochemical compounds respectively.

TABLE II - Regression analysis and one way analysis of variance (ANOVA) of phytochemical composition and antioxidant properties of bean seed

Property	Variety	Regression equation	*R^2	Pred. R^2	**P-value
TEC (g/100g dry wt.)	WB	$TEC = 0.0792X^2 - 0.3221X + 0.636$	0.9725	0.9725	0.00
	RKB	$TEC = 0.1367X^2 - 0.5489X + 0691$	0.9921	0.9254	0.00
	SCB	$TEC = 0.0753X^2 - 0.2746X + 0.612$	0.9225	0.9906	0.00
TPA (g/100g dry wt.)	WB	$TPA = 0.0007X^2 - 0.012X + 0.0614$	0.8411	0.8356	0.00
	RKB	$TPA = 0.011X^2 - 0.0153X + 0.0612$	0.934	0.9205	0.00
	SCB	$TPA = 0.0007X^2 - 0.0139X + 0.0815$	0.7118	0.7067	0.00
TF (g/100g dry wt.)	WB	$TF = 0.0004X^2 - 0.007X + 0.0347$	0.5455	0.8655	0.62
	RKB	$TF = 0.001X^2 - 0.0013X + 0.0487$	0.5563	0.543	0.00
	SCB	$TF = 0.0003X^2 - 0.0093X + 0.068$	0.8672	0.546	0.00
TETAOA (g/100g dry wt.)	WB	$TETAOA = 0.0307X^2 - 0.1785X + 0.494$	0.8889	0.8768	0.00
	RKB	$TETAOA = 0.0573X^2 - 0.3006X + 0.426$	0.9813	0.9761	0.00
	SCB	$TETAOA = 0.0384X^2 - 0.2493X + 0.609$	0.9333	0.924	0.00
IRC (Abs.at 700 nm)	WB	$IRC = 0.0058X^2 - 0.0053X + 0.3084$	0.5965	0.5978	0.47
	RKB	$IRC = 0.0054X^2 - 0.0074X + 0.287$	0.6494	0.7042	0.00
	SCB	$IRC = 0.0069X^2 - 0.014X + 0.2619$	0.7055	0.6539	0.00
LARC (%)	WB	$LARC = 0.3391X^2 - 0.9829X + 20.256$	0.8577	0.8654	0.00
	RKB	$LARC = 0.2622X^2 - 0.4199X + 22.77$	0.827	0.8509	0.00
	SCB	$LARC = 0.3925X^2 - 0.951X + 22.927$	0.8449	0.8318	0.00
HRSC (%)	WB	$HRSC = 0.2181X^2 - 7.1778X + 74.11$	0.7899	0.7879	0.00
	RKB	$HRSC = 0.5616X^2 - 0.1202X + 35.485$	0.974	0.9726	0.00
	SCB	$HRSC = 0.2933X^2 - 3.1876X + 38.468$	0.8362	0.7207	0.00
DPPH RSC (%)	WB	$DPPH\ RSC = -0.4458X^2 + 3.808X + 20.571$	0.5637	0.5669	0.00
	RKB	$DPPH\ RSC = -0.6702X^2 + 5.9405X + 21.505$	0.5745	0.5806	0.00
	SCB	$DPPH\ RSC = -0.6731X^2 + 6.4319X + 18.884$	0.5219	0.5168	0.02

*R^2: Regression coefficient. **p-value show the significant difference between the extracts of a single bean variety in different solvents at the confidence level $p<0.05$ using Tukey's multiple range test. X: Solvent polarity, WB: white beans, RKB, red kidney beans, SCB: small common beans

towards solvent polarity was observed with a high value of regression coefficient (R^2=0.9225-0.9921) for each variety (Figure 2A). Extract yield was found to be comparatively high in water, a polar solvent, than that in nonpolar solvents. The high value of TEC in polar solvent indicates the presence of more polar and water soluble components in bean seeds as compared to non polar ones. TPC and TFC were also found to be polynomial functions of solvent polarity with relatively higher values of R^2 (TPC: 0.700-0.9283 and TF: 0.9437-0.9532) except for TFC in white bean (Figure 2B, C). However, the extracts in nonpolar solvents including hexane and petroleum ether were found to be comparatively high in TPC and TFC of each variety. This indicates a negative correlation between TEC and TPC and TFC of extracts suggesting that phenolic acids are extracted more in nonpolar solvents.

TAOA, IRC and LARC of each variety was found to be increased in polynomial fashion in response to an

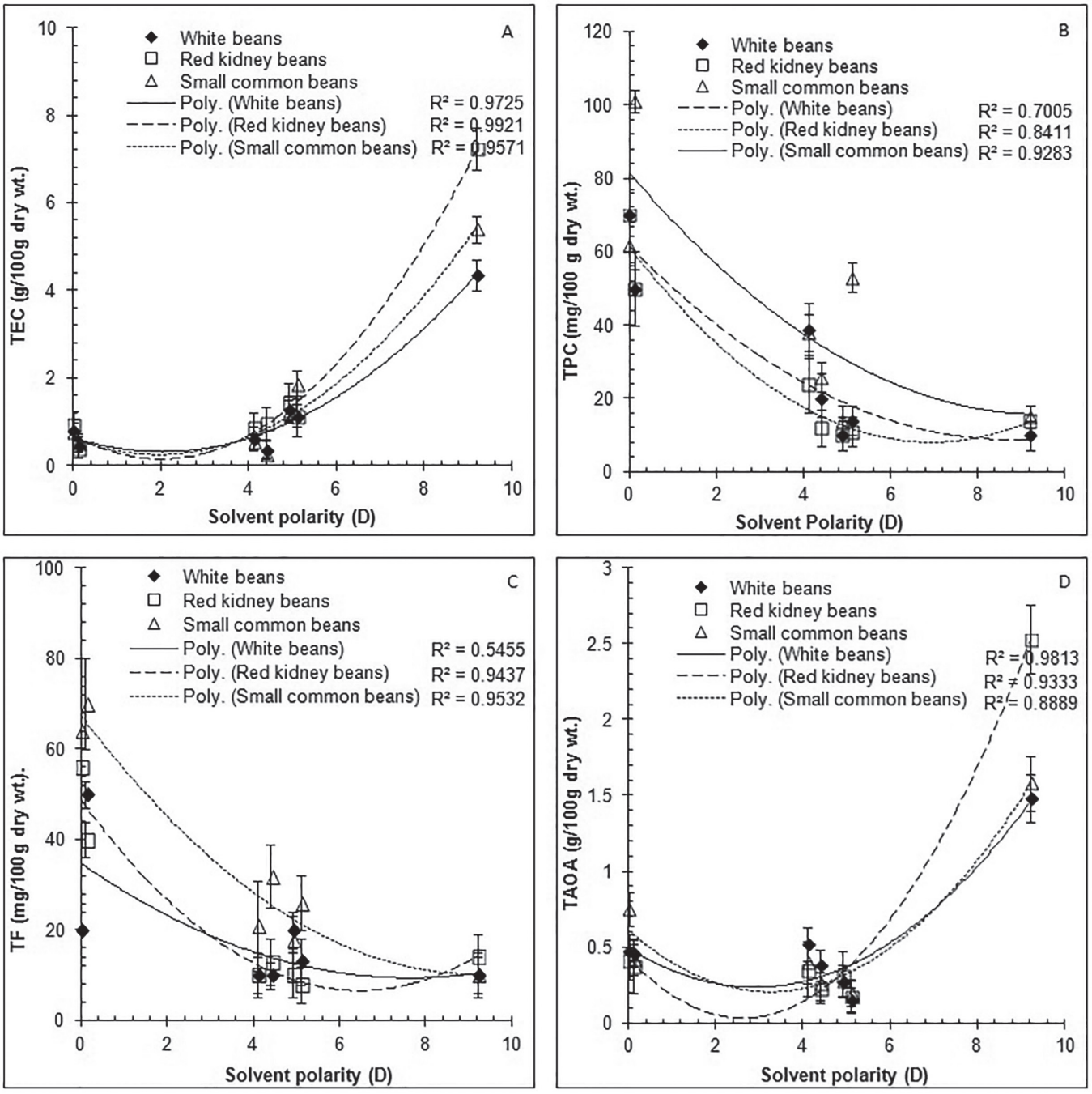

FIGURE 2 - The solvent polarity dependent response of A: total extractable components (TEC), B: total phenolic content (TPC), C: total flavonoid content (TFC) and D: Total antioxidant activity (TAOA) of three varieties of bean seeds.

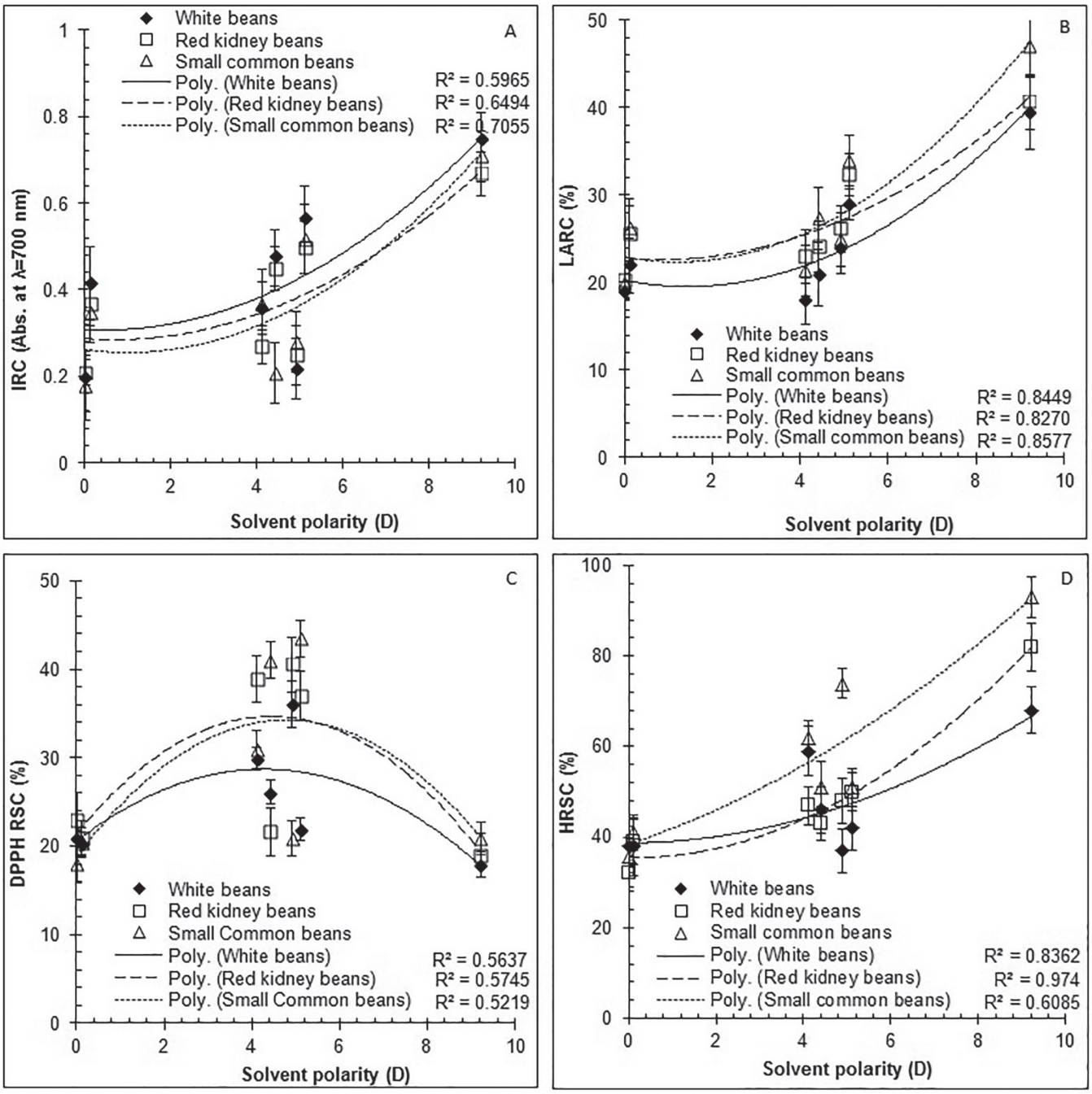

FIGURE 3 - Reducing capacity and free radical scavenging capacity of three varieties of bean seeds at various levels of solvent polarity. A: Iron reducing capacity (IRC), B: Linoleic acid reduction capacity (LARC), C: Hydroxyl radical scavenging capacity (HRSC), D: DPPH radical scavenging capacity (DPPH RSC).

increase in solvent polarity (R^2=0.8889-0.9813, 0.5965-0.7055 and 0.827-0.8577 respectively; Figure 2D, 3A, B). The identical regression curves of TEC and TAOA, IRC and LARC indicate the extraction of more polar components in polar solvents which possess good antioxidant and reducing capacities. Although being rich in phenolic acids and flavonoids, the extracts in nonpolar solvents were found to be poor in antioxidant and reducing properties.

Solvent polarity was also found to exert a polynomial increase in HRSC (R^2=0.6085-0.974) and decrease in DPPH RSC of each variety with relatively low values of R^2 (0.5219-0.5745). Water extract showed a comparatively higher capacity to scavenge hydroxyl radical. However, higher scavenging ability against DPPH radical was shown by bean extracts in solvents of intermediate polarity as compared to polar and nonpolar ones.

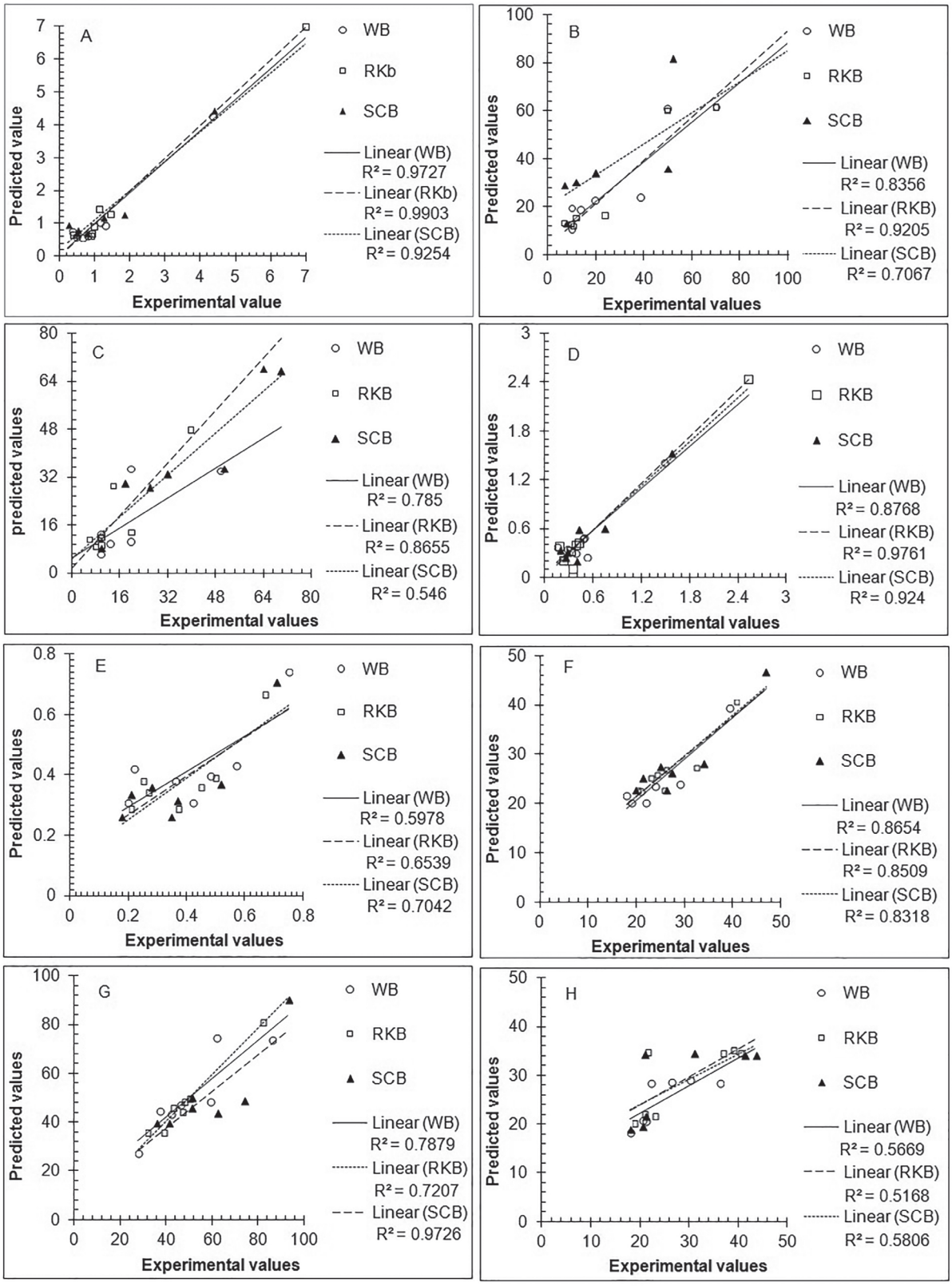

FIGURE 4 - Correlation between experimental and predicted values of phytochemical and antioxidant parameters of Bean seed extracts. A: total extractable components (TEC), B: total phenolic content (TPC), C: total flavonoid content (TFC) and D: Total antioxidant activity (TAOA), E: A: Iron reducing capacity (IRC), F: Linoleic acid reduction capacity (LARC), G: Hydroxyl radical scavenging capacity (HRSC), H: DPPH radical scavenging capacity (DPPH RSC), WB: white beans, RKB, red kidney beans, SCB: small common beans.

The predicted values of response were calculated by putting the values of model terms in the generalized polynomial regression equations and plotted against the experimental values to check the validity of suggested regression model (Figure 4A-H). A good agreement between the experimental and predicted values of TEC, TPC, TF, TAOA, LARC and HRSC was observed indicating the suitability and applicability of the suggested regression model to study the effect of solvent polarity on the extraction of phytochemical and antioxidant properties of bean seeds.

CONCLUSIONS

The polarity-dependent increase in extraction yield, antioxidant activity, reducing properties and free radical scavenging activity of bean varieties may be attributed to the high affinity of antioxidant compounds in bean seeds towards more polar solvents as compared to non-polar ones. However, high values of TPC and TFC in non-polar solvents indicate that most of the phenolic compounds present in bean seeds are non-polar in nature. The results suggest the suitability of polar solvents for the extraction of antioxidant compounds from plant materials particularly bean seeds. The study also suggests that phytochemical compounds extracted in polar solvents are pharmaceutically more important due to comparatively higher values of antioxidant activity, reducing properties and free radical scavenging activity.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest regarding the submitted research article.

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