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ORIGINAL ARTICLE

Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (*Vitis vinifera L*) byproducts

Pooja Doshi · Pandurang Adsule · Kaushik Banerjee · Dasharath Oulkar

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Abstract Grape byproducts are a rich source of phenolics having immense medicinal properties, but usually wasted from juice/wine processing industries. The present study investigates the phenolic antioxidants and the insulinotropic effect of extracts prepared from seed, skin and stems of two red wine grape cultivars: Pusa Navarang and Merlot. Pusa Navarang cultivar has shown high amounts of total phenolics (95.8 mg/ml), flavonoids (30.5 mg/ml) and flavan-3-ols (21.8 mg/ml) in seed extract and total anthocyanin (4.9 mg/ml) in its skin extract as compared to Merlot cultivar. As determined using HPLC, higher amounts of catechin hydrate (14909 mg/l) and epicatechin (9299 mg/l) were observed in its seed extract, while quercetin hydrate (5849 mg/l) was abundant in its skin extract. Similarly, ferric reducing antioxidant power (FRAP) and $ABTS^{+}$. [2,2′-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid] and DPPH. (1,1-diphenyl-2-picrylhy- drazyl) radicals scavenging, were higher in its seed extract, respectively being 134.8 mg/ml of Quercetin equivalent (QE), 18.7 mM of trolox equivalent (TE) and 33.5 mM of TE. Strong correlation was obtained between FRAP and total phenolics, flavonoids and flavan-3-ols contents with correlation coefficients (r^2) of 0.915, 0.738 and 0.838 respectively. Interestingly, there was a 2–8 fold increase in insulin secretion by isolated mice pancreatic islets at 5.5 mM and 16.5 mM glucose concentration in presence of various extracts. Overall, the seed, skin and stem byproducts of both cultivars are rich sources of phenolics and antioxidants and represent a source of new insulin secretagogues.

Keywords FRAP · Free radical scavenging · Total phenolics · Flavonoids · Flavan-3-ols · In vitro insulin secretion

P. Doshi (✉)
Division of Biochemistry, Department of Chemistry,
University of Pune, Ganeshkhind Road,
Pune 411 007 Maharashtra, India
e-mail: pdoshi@chem.unipune.ac.in

P. Adsule · K. Banerjee · D. Oulkar
National Research Centre for Grapes, Pune 412 307 Maharashtra,
India

Introduction

Liquid and solid wastes produced by the food processing industries are increasing now a days. Disposal of these waste materials containing biodegradable organic matter can create environmental problems. Efficient, inexpensive and environmentally rational utilization of agricultural byproducts is of undisputed importance for higher profitability and minimal environmental impact. One of the higher value options is the recovery of bioactive plant food constituents, which could be used in pharmaceutical, cosmetics and food industry (Makris et al. 2007). Grape (*Vitis vinifera*) is one of the world's largest fruit crops with a global production of around 68 million tons in 2008 (OIV 2009). Wine as the main product of this crop reached about 26 million tons in 2008, generating large quantities of waste including grape skins and seeds (OIV 2009). These winery byproducts are rich in high-added-value compounds including phenolic acids, flavanols and anthocyanins (Amico et al. 2008; Bustamante et al. 2008; de Campos et al. 2008; Llobera and Canellas 2008), identified also in grapes and wine (Alonso et al. 2002; Goda et al. 2007; Kammerer et al. 2004; Makris et al. 2007; Spigno and De Faveri 2007; Shrikhande 2000). The grape byproducts either seeds or pomace, constitute a very cheap source for the extraction of antioxidants with potential health promoting and disease protective qualities (Zhang et al. 2007; Louli et al. 2004; Shi et al. 2003), which can be used as dietary supplements, or in the

production of phytochemicals, thus providing an important economic advantage (Alonso et al. 2002; Negro et al. 2003). Phenolic compounds with their antioxidant capacity can preserve flavor and color, avoid vitamin destruction in foods and, more importantly, protect living systems from oxidative damage (Aliakbarian et al. 2009; Moure et al. 2001) and protection against cardiovascular diseases, anti-inflammatory activities and anticarcinogenic effects (Spigno and De Faveri 2007). In addition, antioxidants can be added to the food products containing oil and fat to increase the shelf life of the products (Aliakbarian et al. 2008). In particular, t-resveratrol (trans- 3, 5, 4′-trihydroxystilbene), one of the most important phenolic compounds present in grape, has shown anti-artherosclerosis, anticoronary diseases and anti-cancer properties, which make it particularly attractive for food and human health (Pascual-Marti et al. 2001).

Dietary antioxidants have been associated with the reduced risk of type II diabetes by inhibiting peroxidation chain reactions. Dietary supplements have been used extensively both as pharmacological supplements, food ingredients, in processed foods to aid weight control, and the regulation of glucose control for diabetic patients (Charles 2005). Flavonoids may also have antidiabetic activity (Vessal et al. 2003; Kao et al. 2000; Ong and Khoo 1996; Ahmad et al. 1989). Studies of the in vivo and in vitro effects of various flavonoids on glucose metabolism have shown opposite and often controversial results. This is probably because of the different structural characteristics of the molecules and the different experimental designs used (Harmon and Patel 2003; Kamei et al. 2003; Jarvill-Taylor et al. 2001). The treatment of diabetes mellitus has spent vast amounts of resources in all countries. However, very few studies have investigated the potential of grape pomace as an alternative bioresource for diabetes management (de Campos et al. 2008; Goda et al. 2007; Sehm et al. 2007; Thimothe et al. 2007; Bobek 1999).

Quantitative and qualitative distribution of polyphenols in grape pomace may show significant differences, depending on several factors, such as grape varieties, vinification conditions, the location of cultures and the winemaking procedures (Ruberto et al. 2007). Studies have been carried out in order to try to correlate the variety with the chemical composition of the grapes and wines obtained from them (Dopico-García et al. 2007; Tounsi et al. 2009). However, uses of grape pomace are limited but have been recycled as organic fertilizers, manure, and animal feed (Lafka et al. 2007). For that reason, treatment of winemaking wastes is a serious environmental problem and other uses than as fertilizers have to be found for these by-products. Additionally, little attention has been paid to grape stems, despite that these contain important amounts of polyphenols (Kallay and Kerenyi 1999; Alonso et al. 2002; Doshi et al. 2006; Llobera and Canellas 2007; Makris et al. 2007).

Thus, in the present study the antioxidant capacity and the insulinotropic activity of the phenolic extracts prepared from grape seed, skin and stem of two red wine grape cultivars namely Pusa Navarang and Merlot were determined. The various analyses involved total phenolics, flavonoids, anthocyanin and individual phenolic compounds in the extracts prepared from grape byproducts; seed, skin and stems followed by antioxidant capacity determination in terms of FRAP, ABTS and DPPH assay as well as in vitro insulin secretion by isolated mice pancreatic islets.

Materials and methods

Chemicals

The standard reference chemicals used; (+)-catechin hydrate, gallic acid, quercetin (−) epicatechin, epicatechin gallate, protocatechuic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, rutin hydrate, quercetrin hydrate, trans-resveratrol were obtained from Sigma (St. Lous, MO, USA). Also, other chemicals like 2, 4, 6-tripyridyl-s-triazine (TPTZ), ABTS [2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammoinium salt], DPPH (2, 2-diphenyl-picrylhydrazyl) and Trolox were obtained from Sigma (St. Lous, MO, USA). p-dimethylaminocinnamaldehydde (analytical reagent grade) was obtained from the S.D Fine Chemicals, Ltd, India. All other solvents (HPLC grade) and chemicals used in this study were obtained from Merck, India Ltd.

Sample preparation

The studies were carried out on two colored grape cultivars Pusa Navrang and Merlot (*Vitis vinifera L.*), obtained from vineyard of National Research Centre for Grapes, Pune (M.S) India. The samples were separated into berries and stem (rachis, branches and pedicles) immediately after harvest on the same day. Further, berries were selected from bunches on the basis of their physico-chemical characteristics;., size (12–14 mm), pH (3.5–4.0), TSS (18–20 ° Brix) and titrable acidity (0.7 %–0.8 % tartaric acid equivalent). The skin and seeds were separated from berries pulp. The seeds were air dried and skin and stem samples were subsequently lyophilized using a Freeze Drier (Benchtop 4 K VIRTIS, NY, USA) at −78 °C, powdered and stored at −40 °C for few days till further analyses.

Sample extraction

5.0 g of the different lyophilized samples were extracted by overnight shaking on a mechanical shaker in the dark at RT. The solvent used was 80 % aqueous methanol, as reported

to be a better solvent for polyphenol extraction (Bonilla et al. 2003). The grape skin samples were extracted with 0.01 % HCl in 80 % aqueous methanol. The extracts were centrifuged at 12,000 rpm for 15 min at 4 °C. The residues were re-extracted (2 times, 3 h each) in similar conditions. The filtrates were pooled and concentrated using a rotary evaporator till the methanol gets completely evaporated. The residue was dissolved in 5.0 ml distilled water, filtered through 0.45 μm filters and stored in different aliquots at −40 °C till further analysis.

Spectroscopic analysis of phenolics

Total phenolics, flavonoids, procyanidin monomers (flavan-3-ols) and total anthocyanins contents in the crude extracts were estimated following the previously reported methods (Doshi et al. 2006).

Specific phenolic compounds analysis using HPLC

Separation of individual phenolic components in various extracts was performed on a C-18 column 250 mm x 4.6 mm i.d., 5 μm particle size (Perkin Elmer) using a Dionex system equipped with autosampler (ASI-100), quaternary pump system (P 680) and PDA detector (UVD340U). The mobile phase for chromatographic separation consisted of solvent A: 2 % acetic acid in water v/v, Solvent B: acetonitrile and solvent C: 1 % acetic acid in water and acetonitrile (1:1). The gradients were 0.0–25 min, 88 % solvent A, 2 % solvent B and 10 % solvent C; 38 min, 57 % solvent A, 3 % solvent B and 40 % solvent C; 45–48 min 35 % solvent A, 10 % solvent B and 55 % solvent C; 52–60 min 88 % solvent A, 2 % solvent B and 10 % solvent C. The flow rate was 1.0 ml/min and injection volume was 10 μl of the diluted extracts as per requirement. The identification and quantification of individual phenolics was done by comparison with characteristic spectra, their retention times and calibration curves obtained for the respective external standards.

Antioxidant capacity evaluation

The antioxidant capacity was assessed using one method based on reduction of a ferric tripyridyl-s-triazine complex to its ferrous form, namely FRAP assay (ferric reducing antioxidant power) as described by Benzie and Strain (1996). And two other methods based on measurement of free radical scavenging using ABTS [2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammoinium salt] as reported by Re et al. (1999) and DPPH (2,2-diphenyl-picrylhydrazyl) as reported by Arnous et al. (2001) were also used.

FRAP assay

FRAP assay was carried out following the previously reported procedure (Doshi et al. 2006). In brief, a volume of 100 μl of the diluted samples was added to 1 ml FRAP solution, which was prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM TPTZ in 40 mM HCl) and 2.5 ml of 20 mM ferric chloride ($FeCl_3$). Antioxidant capacity was calculated as the difference in the absorbance at 593 nm after 0 and 4 min (ΔA). The results were expressed as quercetin equivalents (QE) as determined from linear regression obtained by plotting (ΔA) of known quercetin standard solution against concentration (1–5 μg, r^2=0.9982).

Free radical scavenging (antiradical activity)

In the first method, ABTS radical cations ($ABTS^{\cdot+}$) were generated by adding ABTS (7 mM) and potassium persulfate (2.45 mM) in distilled water and kept for 12–24 h in the dark. The ABTS solution was diluted with 50 % ethanol to an absorbance of 0.70 (± 0.05) at 734 nm. 1950 μl of this solution were added to 50 μl of each sample, mixed and absorbance at 734 nm was recorded exactly after 10 min. The suppression of absorbance of $ABTS^{\cdot+}$ Fig. radical cations by sample antioxidants was compared with that of Trolox. Results were expressed as mM Trolox equivalent (TE) as determined from linear regression obtained after plotting absorbance of known Trolox standard solutions against the concentration (100 to 500 μM, r^2=0.9823).

In the second method, 50 μl aliquots of diluted samples were added to 950 μl of $DPPH^{\cdot}$ solution (60 μM in methanol) and the absorbance was read at 515 nm after 30 minutes. The suppression of absorbance of $DPPH^{\cdot}$ radical by sample antioxidants were compared with that of Trolox standard. Results was expressed as mM Trolox equivalent (TE) as determined from linear regression obtained after plotting absorbance of known Trolox standards solutions against the concentration (200 to 600 μM, r^2=0.9862).

Insulin secretion in vitro

Pancreatic Islets isolation

The assay was carried out using mice pancreatic islet following the procedure reported by Shewade et al. (1999), at National Center for Cell Science (NCCS), Pune, India. In brief, Balb/C mice of both sexes (6 to 8 week old) were obtained from an inbred colony maintained at the animal house of NCCS. The islets were purified from a collagenase (Sigma, St Louis, MO, USA) digested pancreas on a Ficoll gradient and cultured in RPMI 1640 media (Gibco, Grand Island, NY, USA) supplemented with 10 % FCS (Trace

Bioscience Pvt. Ltd., New South Wales, Australia) and antibiotics (Penicillin-200 U/ml and Streptomycin—0.2 mg/ml) in culture grade Nunclon flasks at 37 °C, 5 % CO_2 for 48 h.

In vitro insulin secretion assay

The islets were washed twice with Krebs Ringer bicarbonate buffer (pH 7.4) supplemented with 0.5 mg/ml bovine serum albumin (Sigma, St Louis, MO, USA), 1 mM glucose and 10 mM HEPES. The islets were then transferred into 24 well plates (approximately 250 islets per well) and incubated at 37 °C with the same fresh modified Krebs buffer with 0.5 % bovine serum albumin, separately with basal 5.5 mM and 16.5 mM glucose for 1 h. The supernatant was removed and the islets were again incubated at 37 °C with addition of sample extracts at 5.5 mM and 16.5 mM glucose for 1 h. The supernatants collected after both the incubation periods i.e. with and without extracts addition were stored at −65 °C and assayed later by using insulin ELISA kit (Bioscience Europe S.A., Nivelles, Belgium).

Statistics

All experiments were repeated at least three times. The results were analyzed by student's unpaired *t* test and a p value of ≤0.05 was taken to be significantly different.

Results and discussion

Phenolic compounds

The contents of total phenolics, flavonoids and flavan-3-ols (procyanidin monomers) in the extracts of skin, stem and seeds of Pusa Navarang and Merlot grape cultivars obtained were as given in Table 1. The total phenolic content determined in terms of gallic acid equivalents (GAE) was higher in seed extracts as compared to stem and skin extracts, being 65.8 mg/ml in Pusa Navarang cultivar and 41.7 mg/ml in Merlot cultivar. The flavonoids and flavan-3-ol contents as catechin equivalents (CE) were also higher in seed extracts followed by stems and skin of both the cultivars. Grape seeds make about 15 % of solid waste in wine industry and contain approximately (60–70 %) of total extractible grape phenolic compounds (Luque-Rodrıguez et al. 2005; Nawaz et al. 2006). These seeds are an excellent source of monomeric phenolic compounds such as catechin, epicatechin, epicatechin-gallate, dimeric, trimeric and tetrameric procyanidins as well as highly polymerized proanthocyanidins (condensed tannins), which act as anti-aging, anti-mutagenic, anti-proliferative, anti-atherogenic and antiviral agents (Lazzè et al. 2009; Jayaprakasha et al. 2003; Shi et al. 2003a, b). Grape seed extracts were recently reported to have hepatoprotective (Dogan and Celik 2011) and antiallergic properties (Bing-Hung Chen et al. 2012) also.

Table 1 Total phenolics, flavonoids, flavan-3-ols and total anthocyanin contents in seed, skin and berry stem extracts of the two grape cultivars Pusa Navarang and Merlot

Samples	Total phenolics (GAE) mg/ml	Flavonoids (CE) mg/ml	Flavan-3-ols (CE) mg/ml	Total anthocyanin (Cy-3-G) mg/ml
Pusa Navarang				
Seed	65.8±1.26[a]	30.5±0.70[a]	21.8±0.27[a]	NA
Skin	12.5±0.57[b]	2.8±0.11[b]	0.92±0.03[b]	4.9±0.11[a]
Berry stem	11.6±1.99[c]	8.6±0.28[c]	3.7±0.10[c]	NA
Merlot				
Seed	41.7±0.09[d]	26.2±0.70[d]	15.5±0.19[d]	NA
Skin	6.1±0.04[e]	2.9±0.04[b]	0.83±0.03[e]	1.0±0.03[b]
Berry stem	20.5±0.12[f]	14.3±0.31[e]	6.8±0.09[f]	NA

The values are mean ± standard deviation of three replicates and those marked with different letters in same column are significantly different at $p \leq 0.05$

NA Not applicable, *GAE* Gallic acid equivalent, *CE* Catechin equivalent, *Cy-3-G* Cyanidine-3-glucoside equivalent

The total anthocyanin content determined as cyanidine-3-glucoside equivalents (Cy-3-G) was relatively higher in skin of Pusa Navarang cultivar (4.9 mg/ml) as compared to the Merlot cultivar (1.0 mg/ml). This difference could be attributed to purple-red colored skin of Pusa Navarang cultivar and bronze colored skin of Merlot cultivar, which is in agreement with the reported literature that purple skinned grapes have higher anthocyanin content as compared to the bronze skinned grapes (Bonilla et al. 2003). It is also reported that red grape cultivars contain high levels of cyanidin and peonidin in skin tissues, whereas black cultivars contain high levels of delphinidin and malvidin (Shiraishi and Watanabe 1994). Anthocyanin pigments not only contributes towards sensory and organoleptic characteristics as well as antioxidant properties in red grapes and wines (Ghiselli et al. 1998; Pellegrini et al. 2000), but also play an important role in protection towards fungal and bacterial infections (Piermattei et al. 1999).

Specific phenolic compounds

Efforts were made to identify the individual phenolics in the extracts of seed, skin and stem of both grape cultivars using HPLC. Grape phenolic compounds can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids, stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavonols) (Gómez-Alonso et al. 2007). Fourteen phenolic compounds classified as hydroxybenzoic acids: gallic acid, vanillic acid, caffeic acid and syringic acid; hydroxycinnamic acids: protocatechuic acid, chlorogenic acid and p-coumaric acid; flavan-3-ols: catechin, epicatechin,

Table 2 Specific phenolics in the extracts of seed, skin and berry stems of the two grape cultivars Pusa Navarang and Merlot

Grapes	Pusa Navarang			Merlot		
Specific phenolics (mg/l)	seed	skin	berry stem	seed	skin	berry stem
Hydroxybenzoic acids						
Gallic acid	1332.0±90.2 [a]	21.1±2.1[b]	24.3±2.6[b]	689.8±22.0[a]	15.0±2.8[b]	36.1±1.2[c]
Vanillic acid	3.4±1.3[a]	7.5±0.62[b]	76.8±11.6[c]	2.8±0.27[a]	**ND**	66.9±2.5[b]
Caffeic acid	243.7±2.4[a]	52.9±3.9[b]	56.1±7.7[b]	306.7±2.0[a]	48.7±2.1[b]	50.3±0.58[b]
Syringic acid	44.0±2.0[a]	4.9±1.6[b]	5.2±0.18[b]	52.6±3.7[a]	4.8±0.08[b]	8.9±0.52[c]
Hydroxycinnamic acids						
Protocatechuic Acid	78.8±1.2[a]	17.0±0.58[b]	946.4±67.8[c]	87.4±71.0[a]	372.4±45.6[b]	838.5±49.9[c]
Chlorogenic Acid	1013.2±91.6[a]	38.5±3.6[b]	57.9±0.82[c]	258.8±5.7[a]	44.4±2.5[b]	27.1±3.8[c]
p-Coumaric acid	341.3±4.6[a]	**ND**	9.1±2.8[b]	212.2±4.8[a]	2.9±0.53[b]	11.7±0.59[c]
Flavan-3-ols						
catechin hydrate	14909.2±82.3[a]	59.9±0.83[b]	392.5±0.93[c]	3633.9±14.0[a]	885.2±15.5[b]	2380.1±14.7[c]
Epicatechin	9299.4±82.2[a]	34.0±9.5[b]	59.5±0.74[c]	7980.5±2.2[a]	48.8±2.7[b]	85.5±4.5[c]
Epicatechin gallate	1975.1±2.8[a]	21.0±1.3[b]	39.4±2.2[c]	748.9±9.4[a]	26.2±2.7[b]	110.2±1.2[c]
Flavonols						
Quercetrin hydrate	28.7±1.2[a]	5849.7±89.9[b]	56.9±1.5[c]	124.7±1.9[a]	94.9±1.4[b]	85.2±1.7[c]
Rutin hydrate	**ND**	232.3±21.3[a]	**ND**	**ND**	468.4±21.5[a]	119.5±30.5[b]
Stilbenes						
trans-resveratrol	**ND**	34.5±4.9[a]	7.8±9.4[b]	**ND**	37.5±3.6[a]	**ND**
trans-piceatannol	**ND**	5.7±5.8[a]	1521.7±51.6[b]	**ND**	18.9±2.4[a]	**ND**

The values are mean ± standard deviation (SD) of three replicates and those marked with different letters in same column are significantly different at $p \leq 0.05$. ND: not detected

epicatechin gallate; flavonols: quercetrin hydrate and rutin hydrate and stilbenes: trans-resveratrol and trans-piceatannol could be separated using optimized conditions as mentioned in the methodology section. The detection was performed at 280 nm for hydroxybenzoic acids and flavonols; at 320 nm for hydroxycinnamic acids and at 360 nm for flavan-3-ols. Varying levels of different classes of phenolic compounds could be detected from seed, skin and stem of different cultivars as shown in Table 2. Among the hydroxybenzoic acids, gallic acid were detected in high amount in seed extracts of Pusa Navarang cultivar followed by Merlot cultivar respectively being 1332 mg/l and 689 mg/l. Also, caffeic acid and syringic acid was detected higher in seeds as compared to skin and stem. Whereas, vanillic acid content was found to be more in stem extracts of both cultivars respectively being 76 mg/l and 66 mg/l. Among the hydroxycinnamic acids good amount of chlorogenic acid followed by p-coumaric acid could be detected in seeds as compared to skin and stem in both cultivars. The chlorogenic acid was found to be higher in seed extract of Pusa Navarang cultivar (1013 mg/l) as compared to the Merlot cultivar (258 mg/l). Whereas, protocatechuic acid was found to be high in stem extracts of both the cultivars being 946 mg/l in Pusa Navarang cultivar and 838 mg/l in Merlot cultivar. The flavan-3-ols compounds; catechin, epicatechin and epicatechin gallate which are well established for their high antioxidant capacity could be detected in high amounts in seeds followed by stems & least in skin of both the cultivars. The flavonol compounds; quercetrin hydrate having a role in protection & stabilization of color pigment (Chen and

Table 3 Antiradical activity (AR) and ferric reducing antioxidant power (FRAP) in phenolics extracts of seed, skin and berry stems of grape cultivars Pusa Navarang and Merlot

Samples	AR_{ABTS} (TE mM/ml)	AR_{DPPH} (TE mM/ml)	FRAP (QE mM/ml)
Pusa Navarang			
Seed	18.7±0.37[a]	33.5±1.49[a]	134.8±8.46[a]
Skin	4.7±0.01[b]	4.2±0.19[b]	18.6±0.09[b]
Berry stem	4.0±0.12[c]	3.8±0.22[c]	20.6±0.57[c]
Merlot			
Seed	8.0±0.12[d]	11.7±0.11[d]	47.1±2.68[d]
Skin	2.3±0.09[e]	1.8±0.13[e]	5.1±0.48[e]
Berry stem	6.1±0.11[f]	7.9±0.09[f]	29.5±0.72[f]

All the values are mean ± standard deviation of three replicates and those marked with different letters in same column are significantly different at $p \leq 0.05$

AR_{ABTS} Antiradical activity using $ABTS^{+}$. [2,2′-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid] radicals, AR_{DPPH} Antiradical activity using DPPH. (1,1-diphenyl-2-picrylhy- drazyl) radicals, *TE* Trolox equivalent, *QE* Quercetin equivalent

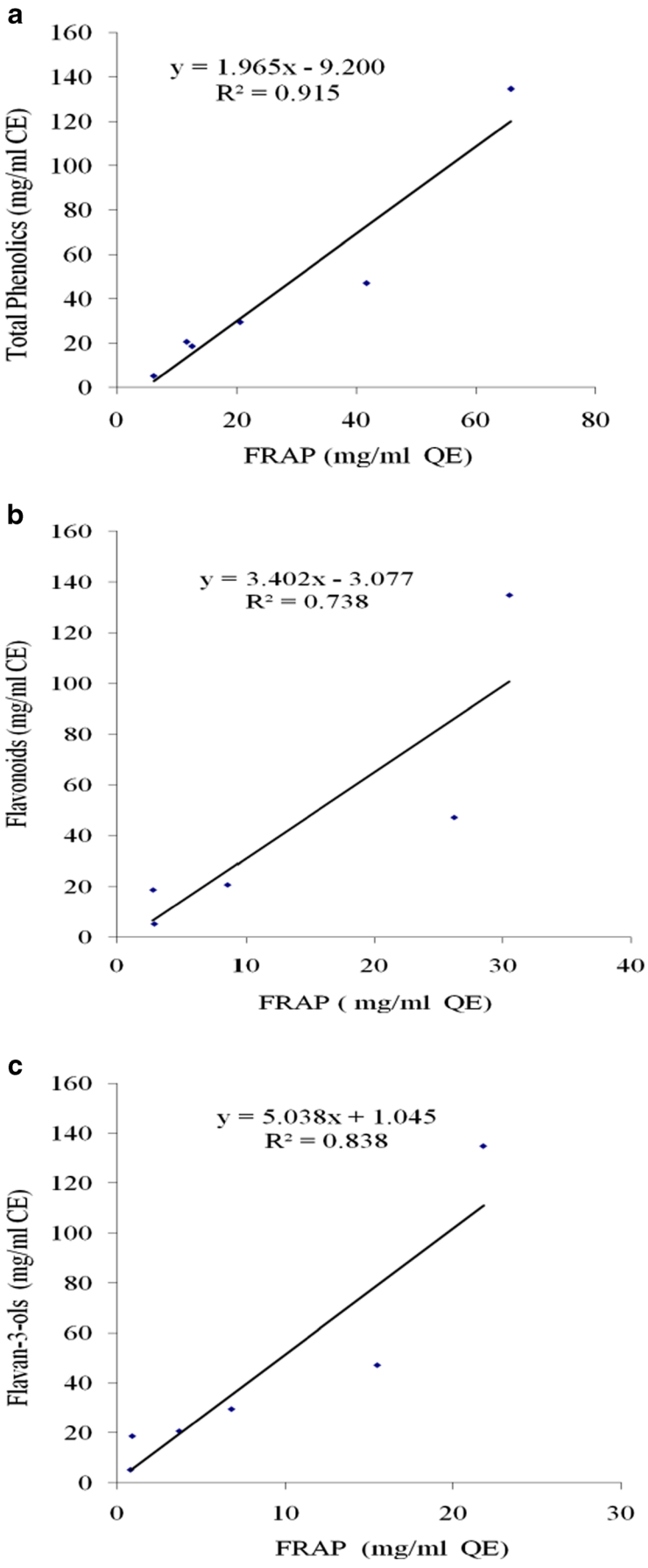

Fig. 1 Correlation of (**a**) Total phenolics, (**b**) Flavonoids and (**c**) Flavan-3-ols with ferric reducing antioxidant power (FRAP) of seed, skin and berry stem of Pusa Navrang and Merlot cultivars. CE: catechin equivalent; QE: Quercetin equivalent

Hrazdina 1981) could be detected in higher amount in skin followed by stem and least in seeds, maximum being 5849 mg/l in skin of Pusa Navarang cultivar. Also, the compound rutin hydrate was higher in skin (468 mg/l) followed by stem (119 mg/l) of Merlot cultivar. Similarly, the stilbene compounds; trans-resveratrol & trans-piceatannol, which are phytoalexins (Jeandet et al. 1991; Hart 1981), could be detected in higher amounts in skin and stem extracts, whereas they could not be detected in seed extracts of both cultivars. The compounds catechin and epicatechin (flavan-3-ols), quercetin and its glycoside rutin (flavonols), and trans-Resveratrol (stilbene) have been proven to be potent antioxidants and to have important biological, pharmacological and medicinal properties (Maier et al. 2009; Villano et al. 2007; Auger et al. 2004; Kammerer et al. 2004; Rice-Evans et al. 1996).

Antioxidant capacity

The results obtained for antioxidant capacity in terms of free radical scavenging (antiradical activity) using $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radicals and FRAP were as given in (Table 3). The maximum antiradical activity was detected in seed extract of Pusa Navarang cultivar being 18.7 and 33.5 mM of Trolox equivalents (TE) respectively for $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radicals. Also, FRAP value was higher in its seed extract, being 134.8 of QE in mg/ml. This result was correlating well with the high content of procyanidin monomers (flavan-3-ols) in the seeds of these cultivars (Table 1). This is in agreement with the reported literature that the flavan-3-ols classified under flavonoid group exhibit substantial antioxidant activity (Cao et al. 1997). Since the majority of medicinal properties proven for the phenolic

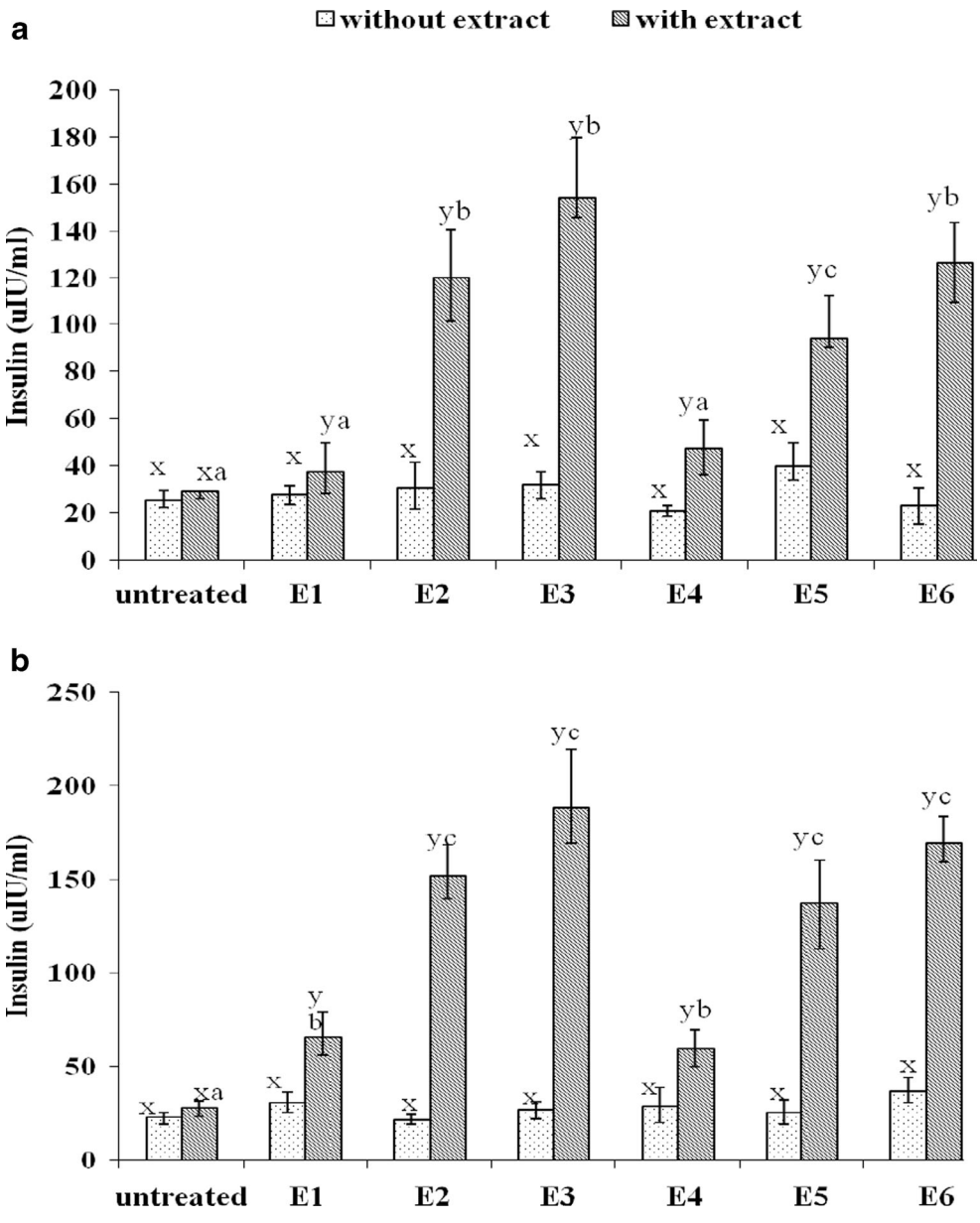

Fig. 2 Stimulation of mice pancreatic islets insulin secretion with extracts of grape seed, skin and berry stems at (**a**) basal glucose level (5.5 mM) and (**b**) enhanced glucose level (16.5 mM) as compared to without extracts.(n=3). E1, E2 and E3 respectively represent extracts of seed, skin and berry stem of Pusa Navarang grape cultivar and E4, E5, E6 respectively represent extracts of seed, skin and berry stem of Merlot grape cultivar. Different letters (a, b, c) on top of bar (mean±SD) indicates significance difference ($p \leq 0.05$), between different parts seed, skin and berry stem and letters x and y indicates significance difference between without extract and with extract respectively

compounds are attributed to the flavan-3-ols and other polymeric procyanidins (Liu et al. 1998; Lotito et al. 2000), this observation is significant from nutraceutical and functional food point of view. So also, the polyphenolic compounds from grape seeds or the alcohol extracts have been reported to show a vitamin E sparing effect (Simonetti et al. 2002) as well as suppression of oxidative stress (Soo-Kyong et al. 2012).

Plotting total phenolics, flavonoids and flavan-3-ols contents with (FRAP), the corresponding correlation coefficients (r^2) obtained were 0.9157, 0.7386 and 0.8385 respectively, as shown (Fig. 1a, b and c). This shows the strong correlation of total phenolic compounds and good correlation of flavonoids as well flavan-3-ols to the antioxidant capacity. This is supported by the reported literature that the antioxidant activity has been highly correlating with the amount of total phenolics and could be attributed to the synergistic effect of overall phenolics composition (Yemis et al. 2008; Meyer et al. 1997). However, the reported literature also suggests that the grape seed extract, usually considered as an antioxidant nutritive supplement, may have prooxidant activity as well, depending on dose, duration of administration, and other dietary components, which was supported by an observation using in vitro primary leukocyte culture, that antioxidant activity of grape seed extract might be mediated by prooxidant quinones and oxidation products of the polyphenols from grape seeds (Chedea et al. 2010)

In vitro Insulin secretion

Interestingly, the extracts prepared from seed, skin and stems of both the grape cultivars have shown insulinotropic effects on isolated mice pancreatic islets. The amount of insulin secreted by islets at different glucose concentration basal (5.5 mM glucose) as well as enhanced glucose concentration (16.5 mM glucose) without extracts and with extracts was represented as (μIU/ml) as given in Fig. 2a and b. Tremendous stimulation in the insulin secretion by a set of pancreatic islets was observed with the presence of crude extracts of all the samples as compared to the insulin secreted by the same set of islets without the extracts at both glucose concentrations. The percentage increase in insulin secreted by mice pancreatic islets in presence of various extracts as compared to the insulin secretion by islets without extracts was in the range of 135 % to 543 % at 5.5 mM glucose concentration as given in Fig. 2a. Whereas, the insulinotropic effect of all the extracts were better evident at higher glucose concentration (16.5 mM) with increase in the range of 206 % to 807 % as compared to the islets without extracts as given in Fig. 2b.

According to the reported literature, the extracts of a traditional antidiabetic plant in India *Asparagus adscendens* (Shweta musali) have shown 19–248 % increase in insulin secretion by clonal pancreatic β cell line, BRIN-BD11 (Mathews et al. 2006). The another traditional antidiabetic plant *Teucrium polium* extracts have shown 135 % increase in insulin secretion by isolated rat islets at 16.0 mM glucose concentration (Razieh et al. 2005). Also, the well documented traditional antidiabetic plant *Viscum album* (mistletoe) has shown 1.1 to 12.2 fold stimulation in insulin secretion by clonal pancreatic β-cell at 16.7 mM glucose concentration (Gray and Flatt 1999). Thus, in the present study the 2 to 8 fold stimulation in insulin secretion by isolated pancreatic islets at 16.5 mM glucose concentration in presence of grape seed, skin and stems extracts is highly appreciating and represents a source of potential new oral hypoglycemic agent.

The grape seed extracts are well established as potent antioxidants and the recent literature has also reported that grape seed extracts have antihyperglycemic and insulinomimetic properties as the grape seed procyanidins mimic or influence insulin effects by directly acting on specific components of the insulin signaling transduction pathway (Meeprom et al. 2011; Pinent et al. 2004). The grape pomace extracts exerted a significant anti-postprandial hyperglycemic effect, suggesting that grape pomace could be a valuable food derived bioresource that is rich in antioxidants and anti-hyperglycemic compounds. These dual bioactive attributes derived from the grape pomace could play a complementary and alternative role in managing the poorly regulated blood glucose levels and oxidative stress associated with Type 2 diabetes (Pinent et al. 2004; Al-Awwadi et al. 2004). Also, grape skin extract has been recently reported to improve glycemia and inflammation (Shelly et al. 2011). Though the flavan-3-ol (procyanidin) contents were relatively less in grape skin and stem the higher insulin secreting activity could be attributed to the other specific phenolic components like flavonols and stilbenes present in abundant in these grape parts. Also, the higher insulinotropic effect of grape skin could also be attributed to anthocyanin pigments which are reported to have property of insulin secretion when exposed to pancretic β-cells (Jayaprakasam et al. 2005). However, to our knowledge this is the first report of an insulinotropic effect of grape stem extracts.

Conclusion

In conclusion, our results suggest that besides grape seeds the grape skin and stem extracts not only have the high antioxidant properties but also have the immense potential as insulin secretagogues and may be useful in the treatment of type II diabetes. However, in vivo studies and clinical evaluations of these compounds must be carried out to further validate our in vitro results. Also, exploration of these grape tissues awaits further research and purification of the active components from the crude extracts. Future work assessing its use as a dietary adjunct or as a source of active components may provide new opportunities for the treatment of diabetes.

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