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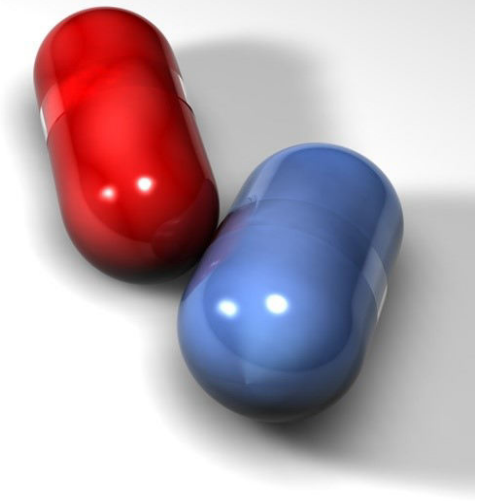

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Letter to the Editor

Correlation between the total phenolics, proanthocyanidins content and anti-oxidant activity of grape seed extract

Sir,

The therapeutic properties of grape seed extract are invaluable assets to human beings. A large number of diseases such as arthritis, atherosclerosis, hemorrhagic shock, advancing age, Alzheimer and Parkinson's disease, gastrointestinal dysfunctions, tumor promotion and carcinogenesis are associated with free radicals accumulation (Bagchi et al., 2000). Hence, it is important to prevent the accumulation of free radicals in cells. Many natural products including tea, olive oil and cocoa were reported to possess high anti-oxidant activity (Kay et al., 2006). Another important natural product rich in antioxidant activity is grape seed. Gulabi variety of grape is extensively cultivated in southern part of India. Hence, the aim of this research is to determine the antioxidant activity of ethanolic extract of gulabi grapes (*Vitis vinifera*) and correlate it with total phenolics and proanthocyanidins content.

Solvent extraction was followed to prepare grape seed extract (Li et al., 2008). The total phenolic content of grape seed extract was determined by the Folin-Ciocalteau method using gallic acid as a calibration standard. The absorbance was read at 750 nm after 30 min of reaction. The final results were usually expressed as gallic acid equivalent per gram of grape seed extract (mg/g). The estimation of total phenolic contents was conducted in triplicate. The total extractable proanthocyanidins present in the grape seed extract was determined from diluted (1:4 v/v) grape seed extract (filtrate of extraction suspension containing 0.5 g of grape seed extract with 20 mL of 50% ethanol kept at 80°C in a water bath (OLS200 Biolinx, India) under shaking condition (200 rpm) for 3 hours in two repetitions) according to the optimized procedure of Ana Bucić-Kojic et al. (2008). The results were usually expressed as cyaniding equivalent per gram of grape seed extract. The antioxidant activity of the grape seed extract was determined using two different assays: DPPH and ABTS assay (Yusoff et al., 2015). The percentage of scavenging activity by sample and control is given by the following formula:

$$\% \text{ radical scavenging activity} = \left(\frac{Ab - As}{Ab}\right) * 100$$

Where Ab denoted the absorbance of blank and As denoted the absorbance of sample

IC_{50} value was obtained by interpolation from linear regression analysis. In ABTS assay, Trolox equivalent anti-oxidant activity of the samples was obtained from Trolox standard curve and it was expressed as mM of Trolox equivalent per g of grape seed extract. All results were expressed as mean ± standard deviation.

The total phenolic content of grape seed extract was obtained from linear regression equation of gallic acid standard calibration curve ($y = 0.0012x + 0.0054$, $R^2 = 0.9984$). The amount of phenolic materials present in the grape seed extract was found to be 226 ± 4.3 mg gallic acid equivalent per gram of grape seed extract. The amount of proanthocyanidins present in the grape seed extract was found to be 24.8 ± 1.4 mg cyanidin equivalent per gram of grape seed extract.

As the concentration of grape seed extract increased, the DPPH radical scavenging activity also increased (Figure 1a). Hence, the different concentrations of sample scavenged the DPPH radicals in a concentration –dependent manner. The highest scavenging of DPPH (85.4 ± 5.8%) occurred at the grape seed extract concentration of 25 mg/mL. The IC_{50} value of the grape seed extract and quercetin standard was found to be 8.0 mg/mL and 0.009 mg/mL. The lower the IC_{50} value, the higher the ability to scavenge DPPH radicals. In this case, grape seed extract had low DPPH radical scavenging activity when compared to the quercetin standard.

Another important antioxidant assay is the ABTS cation decolorization assay. In this assay, the antioxidant activity was measured as Trolox equivalent anti-oxidant capacity. The standard used in this assay was trolox, which is a known antioxidant agent. Analog to the DPPH radical scavenging activity, the sample scavenged the ABTS radical in a concentration –dependent fashion at the concentration range of 0.25 to 2 mg/mL (Figure 1b). The highest activity (21.8 ± 2.7%) was measured at grape seed extract concentration of 2 mg/mL. The IC_{50} value of the sample and Trolox standard was determined as 4.8 and 1.5 mg/mL. When compared to DPPH radicals, ABTS radicals were scavenged well at the lower concentration of grape seed extract.

The correlation coefficient (r) values were estimated between antioxidant assays (DPPH and ABTS) and the phenolic content (total phenolic contents and total

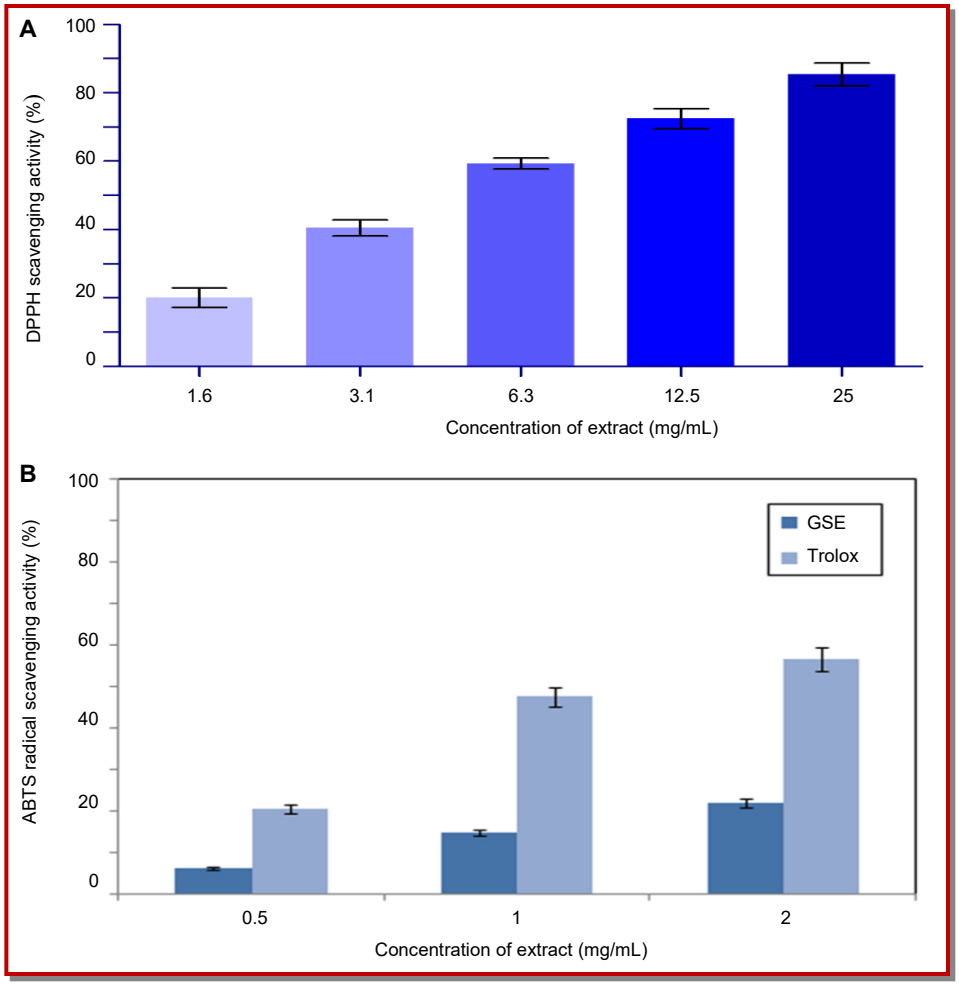

Figure 1: Radical scavenging activity of grape seed extract determined by DPPH assay (A) and ABTS assay (B)

Table I

Correlation coefficients obtained between anti-oxidant assay, total phenolics and proanthocyanidins content

	TPC[a]	TPA[b]	DPPH[c]	ABTS[d]
TPC	-	0.9950*	0.8008§	0.9579
TPA	0.9950*	-	0.7935§	0.9440
DPPH	0.8008§	0.7935§	-	0.9444
ABTS	0.9579	0.9440	0.9444	-

§ and * indicates $p<0.05$ and $p<0.01$, [a]TPC, total phenolic content; [b]TPA, total extractable proanthocyanidins; [c]DPPH, DPPH radical scavenging activity; [d]ABTS, ABTS radical scavenging activity

extractable proanthocyanidins) of the grape seed extract. The positive linear correlation was found between these two parameters (Table I). The correlations between the total phenolic contents and the assays (DPPH and ABTS) were found to be 0.8008 and 0.7935. Meanwhile, the correlations between the total extractable proanthocyanidins and the assays (DPPH and ABTS) were found to be 0.9579 and 0.9440.

In conclusion, a positive correlation was observed between total phenolics, proanthocyanidins content and antioxidant activity of grape seed extract.

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