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EVALUATION OF ANTIOXIDANTS IN THE SEED FLOUR OF GULABI VARIETY OF *Vitis vinifera*

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ABSTRACT

Gulabi variety of Vitis vinifera is grown in Karnataka, mainly used for making juice and wine. Vitis vinifera seed oil is produced from the seeds in the pomace left over from juice and wine production, and thus adds value to the industry. Vitis vinifera seed flour, the residue from seed oil manufacture, has not received much attention but may be a potential rich source of natural antioxidants and other healthful bioactive compounds. Thus this research focus on the study of antioxidant present in this flour.

The protein content in the crude extract of cold water, hot water, methanol and ethanol by using BSA (1mg/ml)as standard and the protein content was found to be 28.7, 30.6, 32.3 and 25.8mg of BSAE/g of seed respectively. The phenolic content in the crude extract of cold water, hot water, methanol and ethanol were found out to be 0.024, 0.043, 0.04 and 0.043 mg GAE per gram of seed, respectively. For hydroxyl free radical scavenging, the maximum inhibition was obtained for methanolic extract at 60 minutes followed by the ethanolic extract of the same. The hot water extract of V.vinifera gave maximum result at 30 minutes, wherein the cold water extract gave steady inhibition throughout The antioxidant content in the crude extract of cold water, hot water, methanol and ethanol were found out to be 8.71, 10.35, 11.59 and 9.77 mg AAE per gram of seed, respectively for V.vinifera. Based on the % inhibition of free radical, for V.vinifera , the maximum inhibition was observed for cold water and for alcoholic solvents , methanolic extract has more antioxidants than ethanolic extract. The amount of catalase in the crude extract of cold water, hot water, methanol and ethanol were found out to be 7.16, 5.58, 5.14 and 3.86 units of enzyme per gram respectively for V.vinifera.

Keywords: Gulabi, Vitis vinifera, antioxidant, hydroxyl free radical, catalase

I INTRODUCTION

Vitis vinifera are mainly utilized for their juice, which is used primarily in wine-making. *Vitis vinifera* seed oil is produced from the seeds in the pomace left over from juice and wine production, and thus adds value to the industry. *Vitis vinifera* seed flour, the residue from seed oil manufacture, has not received much attention but may be a potential rich source of natural antioxidants and other healthful bioactive compounds (Luther et al., 2007). Gulabi variety is grown in Karnataka. Berries are small sized, dark purple, ovoid, seeded with thick skin. Juice is purple coloured, clear and pleasantly flavoured with 16-18% TSS. Variety has a good keeping quality and mainly used for making juice and wine. *Vitis vinifera* seed proanthocyanidins were found to induce apoptosis and inhibit metastasis in both cultured breast and colon cancer cells (Mantena, Baliga, & Katiyar, 2006). In 2007, it was reported that an ethanol extract of chardonnay *Vitis vinifera* seed flour not only suppressed overall lipid peroxidation in fish oil, but also protected eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the longer bioactive n-3 fatty acids, against oxidative loss (Luther et al., 2007). These findings were supported by the observations of a recent finding that *Vitis vinifera* seed residues from mechanical oil extraction were rich in polyphenolics with strong antioxidant properties (Maier, Schieber, Kammaerer, & Carle, 2009). These previous studies suggested the potential of developing natural antioxidative preparations from *Vitis vinifera* seed flours for shelf-life enhancing applications in foods. Lipid peroxidation is a major problem of food quality, safety and nutritional value. Lipid peroxidation leads to off-flavour development in foods and reduces the nutritional and economic value of food products. Antioxidants are often added to food formulae to increase their oxidative stability and prolong shelf life. Many of the antioxidants used in the food industry are chemically synthesised: mainly butylated hydroxyl anisole, butylated hydroxyl toluene (BHA and BHT, respectively) and propyl gallate (Singh, Marimuthu, de Heluani, & Catalan, 2005). Natural antioxidants derived from edible materials are in high demand for food applications amidst concerns over the safety of the consumption of these synthetic antioxidants (Ito, Fukushim, & Tsuda, 1985).

II MATERIALS AND METHODS

MATERIALS

Chemicals and reagents used such as α-amylase, gallic acid, α -D Glucose, Sodium phosphate buffer, Folin-Ciocalteau's reagent, Lowry's reagent, Dinitrosalicylic acid, were procured from Sigma-Aldrich,sodium carbonate, Methanol, Ethanol, starch were of analytical grade while the water was glass distilled.

SAMPLES

The seeds of Banglaore blue variety of *Vitis vinifera* were purchased from a local market in Chennai, India. The seeds were hand selected to eliminate those that were cracked. The seeds were then dried at 45°C for 30 minutes. It was then stored in an airtight container till use.

DEFATTING OF SEEDS

Defatting was carried out according to the mechanical cold expeller method (El-Adawy and Taha, 2001) but with a slight difference.10 g of seeds stored were homogenized. The fat was separated using 50 ml n-Hexane and was filtered using Whatman no.1 filter paper. The sample in powdered form collected on the filter paper was stored for the extraction. The filtrate collected was stored for the lipid analysis. The defatted sample was used for all the analysis.

PREPARATION OF SEED EXTRACTS

The seed extracts were prepared using four different solvents such as cold water (28°C), hot water (45°C), Methanol and Ethanol. 1g of the sample was mixed with 20 ml of cold water (28°C) .The filtrate was collected and labelled as sample (C). The same procedure was repeated for the other three solvents and were labelled as sample (H), (M) and (E) for hot water methanol and ethanol respectively.

TOTAL CRUDE PROTEIN ESTIMATION

The total crude protein was estimated using Lowry's method. Appropriate dilutions of the seed extracts were added to 2ml of Lowry's reagent [(a) 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (0.4 gm in 100 ml distilled water.) (b) 10 ml of 1.56% copper sulphate solution mixed with 10 ml of 2.37% sodium potassium tartarate solution. Prepare analytical reagents by mixing 2 ml of (b) with 100 ml of (a) and mixed well. It was incubated for 10 minutes at room temperature.0.2ml of Folins Ciocalteau solution was the added to it and incubated for 30 minutes. The absorbance was then taken at 660nm. A plot of Absorbance against Protein concentration was made to get a standard calibration curve. From the curve, the amount of total protein was estimated.

DETERMINATION OF TOTAL PHENOLIC CONTENT

Total free phenolics were estimated using Folin-Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, appropriate dilution of the seed extracts were oxidized with 2.5 mL 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45 °C and the absorbance was measured at 725 nm in the UV-Visible spectrophotometer. Then, the total phenol content was subsequently calculated as gallic acid equivalents.

HYDROXYL FREE RADICAL SCAVENGING ASSAY

The bioassay was performed according to a previously described procedure (Axelrod, Cheesbrough, & Laakso, 1981) 1.5 mL of different extracts was mixed with 0.02 mL of 30% of H_2O_2 solution. Absorbance was read at 530 nm at different times (5–60 min). Decreased absorbance of the reaction mixture indicated increased in scavenging ability. The percentage of inhibition of H_2O_2 radical is calculated using the following equation:

% Inhibition of H_2O_2 = ((Abs $_{control}$ –Abs $_{extract}$) / Abs $_{control}$) *100

Where, Abs $_{control}$ is the absorbance of the control (without H_2O_2) and Abs $_{extract}$ the absorbance in the presence of the extracts, then the time required to inhibit 50% (IT50) of H_2O_2radical was determined.

EVALUATION OF TOTAL ANTIOXIDANT CAPACITY

It was determined according to the method of Prieto et al (1999); 0.3 ml of sample solution containing reducing species was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples. For samples of unknown composition, antioxidant capacities were expressed as equivalents of ascorbic acid.

DPPH FREE RADICAL SCAVENGIGNG ASSAY:

This method was performed according to the method of Payet, Shum Cheong Sing and
Smadja (Payet, Shum Cheong Sing, & Smadja, 2005), with modifications. 200µLl of extract (1mg/ml) was added to 2.8ml of 0.001% methanolic DPPH. This mixture was incubated in dark for 15 minutes. Using 200µl in 2.8ml DPPH as control, the absorbance was measured at 492nm.

% inhibition= ((Abs $_{control}$ –Abs $_{extract}$) / Abs $_{control}$) *100

CATALASE ASSAY

3ml of 0.067M H2O2-phosphate buffer (pH-7) was added to 40µl of enzyme extract in the experimental cuvette and mixed thoroughly. The time required for the decrease in absorbance by 0.05 units was recorded at 240nm in a spectrophotometer. The absorbance was measured for different times (5- 60 min).The enzyme solution containing H2O2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

III RESULT

LOWRY'S METHOD

The protein content in the crude extract of cold water, hot water, methanol and ethanol by using BSA (1mg/ml)as standard and the protein content was found to be 28.7, 30.6, 32.3 and 25.8mg of BSAE/g of seed respectively. The results are presented in Figure 1.The total polar protein that dissolves in cold water, hot water, methanol and ethanol was found to be very less.

DETERMINATION OF TOTAL PHENOL CONTENT

The total phenolic content was determined in each extract since it is considered as major determinant of the antioxidant activity of nuts and plants (Kris-Etherton et al., 2002). Gallic acid(1mg/ml) was used as standard The results are presented in Figure 2. The phenolic content in the crude extract of cold water, hot water, methanol and ethanol were found out to be 0.024, 0.043, 0.04 and 0.043 mg GAE per gram of seed, respectively for *V.vinifera*. The high concentration of phenolics in the water extract can be attributed to the presence of proteins and other water soluble constituents that contain phenolic rings.

HYDROXYL FREE RADICAL SCAVENGING ASSAY

The hydroxyl radical can damage virtually all types of macromolecules: carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation) and amino acids (e.g. conversion of Phe to m-Tyrosine and o- Tyrosine). The hydroxyl radical has a very short *in vivo* half-life of approximately 109 seconds and a high reactivity. This makes it a very dangerous compound to the organism. Unlike superoxide, which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction. Mechanisms for scavenging peroxyl radicals for the protection of cellular structures include dietary antioxidants such as flavonoid and vitamin C. The hydroxyl radical scavenging activity of the various extracts was investigated (Figure 3).

The maximum inhibition was obtained for methanolic extract at 60 minutes followed by the ethanolic extract of the same. The hot water extract of *V.vinifera* gave maximum result at 30 minutes, wherein the cold water extract gave steady inhibition throughout.

The scavenging activity of the extract was more in cold water and ethanolic extract, wherein methanolic extract and hot water gave a steady scavenging activity. Based on the finding, presence of phenolic group affects the antioxidant activity.

EVALUATION OF TOTAL ANTIOXIDANT CAPACITY

The total antioxidant content was by the modified method described by Prieto et al (1999);. Ascorbic acid(1mg/ml) was used as standard The results are presented in Figure 4. The antioxidant content in the crude extract of cold water, hot water, methanol and ethanol were found out to be 8.71, 10.35, 11.59 and 9.77 mg AAE per gram of seed, respectively for *V.vinifera*.

DPPH FREE RADICAL SCAVENGIGNG ASSAY:

The DPPH radical scavenging activity of extract are presented in figure 4. The DPPH free-radical scavenging activity ranged from 29.26 to 58.5%. Based on the % inhibition of free radical, for *V.vinifera* , the maximum

inhibition was observed for cold water and for alcoholic solvents , methanolic extract has more antioxidants than ethanolic extract.

CATALASE ASSAY

The amount of catalase in the crude extract of cold water, hot water, methanol and ethanol were found out to be 7.16, 5.58, 5.14 and 3.86 units of enzyme per gram respectively for *V.vinifera*. Presence of catalase activates many reactions. If there is more catalase in the extract, the antioxidant activity is greater.

IV CONCLUSION

The protein content in the crude extract of cold water, hot water, methanol and ethanol by using BSA (1mg/ml)as standard and the protein content was found to be 28.7, 30.6, 32.3 and 25.8mg of BSAE/g of seed respectively. The phenolic content in the crude extract of cold water, hot water, methanol and ethanol were found out to be 0.024, 0.043, 0.04 and 0.043 mg GAE per gram of seed, respectively. For hydroxyl free radical scavenging, the maximum inhibition was obtained for methanolic extract at 60 minutes followed by the ethanolic extract of the same. The hot water extract of *V.vinifera* gave maximum result at 30 minutes, wherein the cold water extract gave steady inhibition throughout. The antioxidant content in the crude extract of cold water, hot water, methanol and ethanol were found out to be 8.71, 10.35, 11.59 and 9.77 mg AAE per gram of seed, respectively for *V.vinifera*. Based on the % inhibition of free radical, for *V.vinifera* , the maximum inhibition was observed for cold water and for alcoholic solvents , methanolic extract has more antioxidants than ethanolic extract. Based on the finding, presence of phenolic group affects the antioxidant activity.

The amount of catalase in the crude extract of cold water, hot water, methanol and ethanol were found out to be 7.16, 5.58, 5.14 and 3.86 units of enzyme per gram respectively for *V.vinifera*.

Based on the study done, it can be found that the seed extract of Gulabi varieties of *V.vinifera* has a good antioxidant activity. Based on the phenolic content and H_2O_2 inhibition assay, the antioxidant activity of the extract was confirmed. Based on results, it was found that the cold water and methanolic extract of seeds of Gulabi varieties of *V.vinifera* were found to be more effective when compared with the alternate extract.

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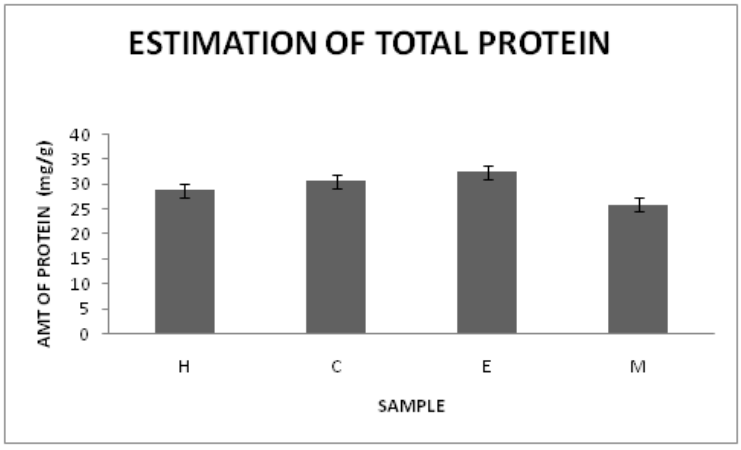

Figure 1: ESTIMATION OF PROTEIN IN THE EXTRACT

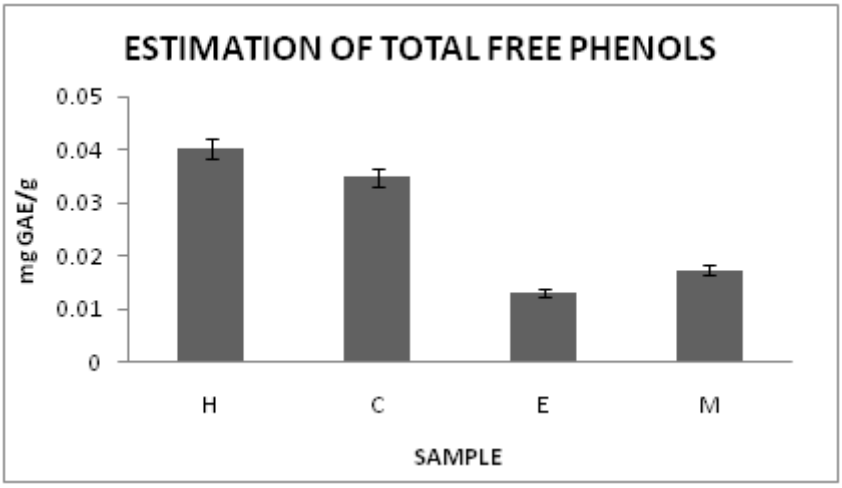

Figure 2: ESTIMATION OF TOTAL FREE PHENOLICS

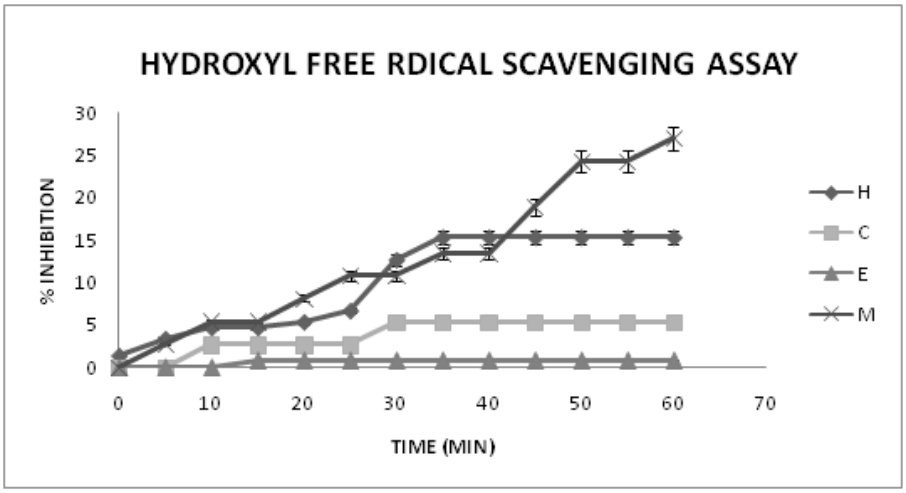

Figure 3: HYDROXYL FREE RADICAL SCAVENGING

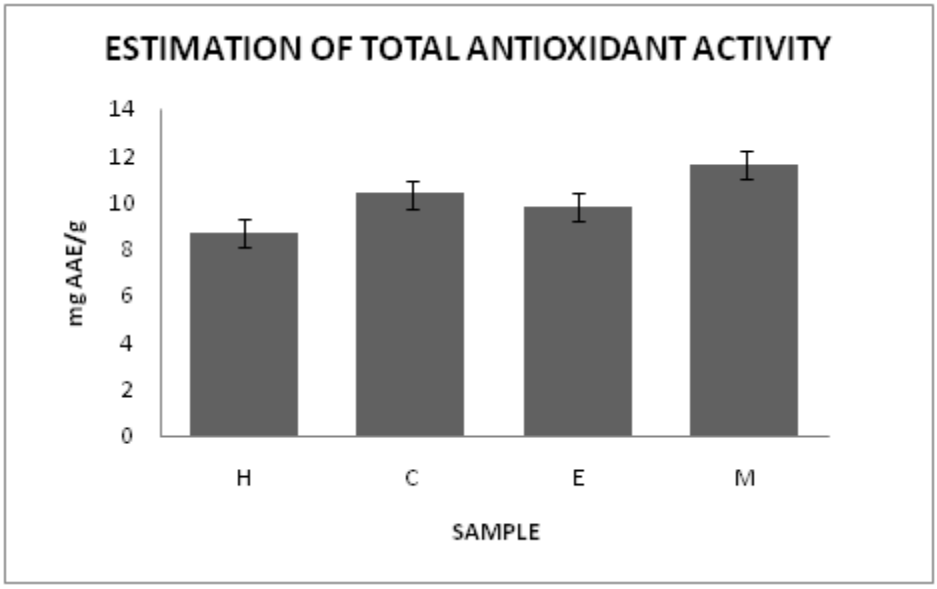

Figure 4: ESTIMATION OF TOTAL ANTIOXIDANT CAPACITY

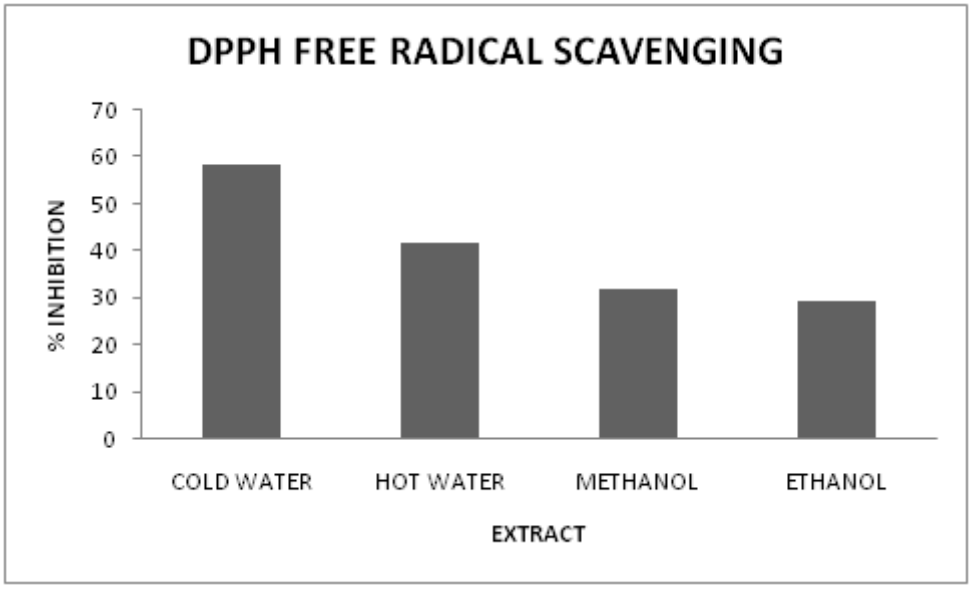

FIGURE 5: DPPH FREE RADICAL SCAVENGING

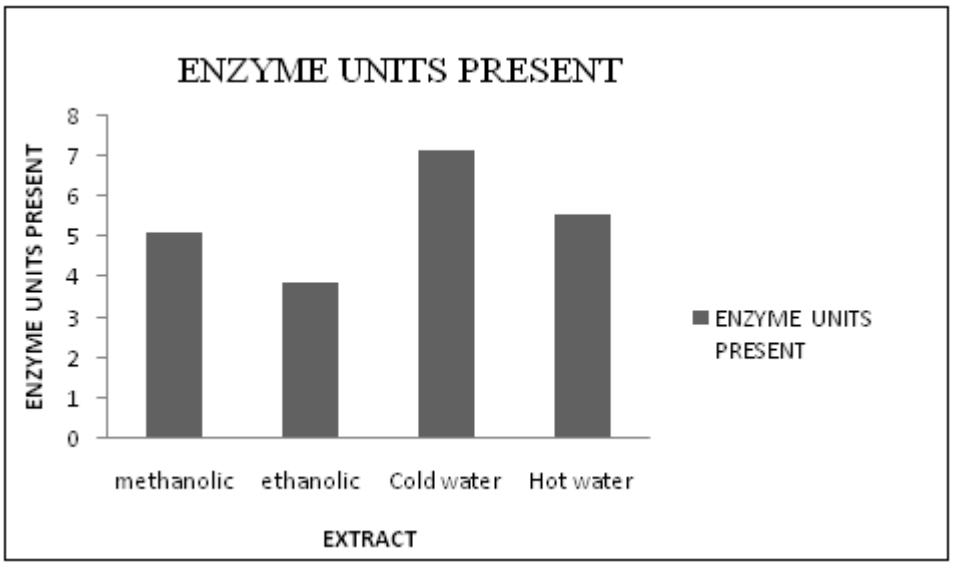

FIGURE 6: ENZYME UNITS PRESENT