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In vitro Antioxidant Activities of Green and Black Grape (*Vitis vinifera* L.) Methanolic Extracts- A Comparative Study

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ABSTRACT: The skin and seeds of grapes are known as a rich source of antioxidants and dietary intake of foods rich in antioxidants are suggested to be cancer protective. In present study we have made a comparative account on antioxidant activities of green and black grape (*Vitis vinifera* L.) methanolic extracts. The antioxidant activities were determined by using DPPH (2,2'-diphenyl-1-picrylhydrazyl) assay. According to the results of present screening study, green grape methanolic extract posseses high antioxidant activity giving IC_{50} value 6.42µg/ml as compared to black grape methanolic extract giving IC_{50} value 7.31µg/ml.

Keywords: Antioxidant activity, Green and black grapes, *Vitis vinifera* L., DPPH assay, IC_{50} Value.

I. INTRODUCTION

The grape berries are important since they are consumed as fruits, wine, juice or raisins and are largely cultivated for the wine industry. Grapes contain a wide range of chemical substances such as sugars, organic acids, mineral salts, vitamins, enzymes and also phytochemicals which are responsible for the sensory characteristics of wines [20] and for their health properties [19]. The use of synthetic antioxidants in the food has been under scrutiny for toxicological reasons, and therefore the interest in the natural antioxidants has steadily been increasing [3, 14, 5]. The antioxidant and radical scavenging activities of a large number of polyphenolic compounds isolated from plants have been studied [7, 15, 13, 22, 8]. The main classes of natural antioxidant compounds in nature are flavonoids and phenolic acids in free or complexed forms. These compounds have been identified and quantified in several fruits and vegetables, and show a high correlation with antioxidant activity [12, 18]. Grapes and wine contain high amounts of phenolic compounds, mainly flavonoids. Most phenolic compounds found in wine can act as antioxidants [21]. Likewise, residues of wine production are also characterised by high contents of phenolic compounds due to an incomplete extraction during wine production. Furthermore, phenolic constituents present in grapes have aroused great interest due to their antioxidant properties and putative health-promoting effects. Polyphenolic compound in grapes are known to lower oxidative stress [4], to modulate the inflammatory cascade [9], to reduce the oxidation of LDL-c [16] and to induce protection against atherothrombotic episodes including myocardial ischemia and inhibition of platelet aggregation [2,17]. Most of these health effects have been ascribed to polyphenolic compounds serving as reducing agents in many biological systems by donating hydrogen, quenching singlet oxygen, acting as chelators and by trapping free radicals. Moreover, these antioxidant activities help to limit oxidation of nucleic acids, proteins, lipids, which may initiate degenerative diseases such as cancer, heart disease, dermal disorders and aging [10,11].

In this present study we are comparing the antioxidant activities of green and black grapes using DPPH radical scavenging activity.

II. MATERIALS AND METHODS

A. Materials and Chemicals

The berries of green and black grapes (*Vitis vinifera* L.) were collected from farms of Nasik and were brought to lab and then washed and were further processed for extraction.

The following standards and reagents were used: Stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl), methanol.

B. Extraction

Extraction was done by using maceration process. Green Grape berries (500gm) were grounded and dipped in solvent (methanol) and the mixture was left for four days with occasional shaking or stirring. The extract was then taken out and allowed to dry in oven. This process was repeated with the left over grounded residue of berries until the solvent runs clear. Same process for extraction was done with Black grape berries.

Evaluation of antioxidant activity. Evaluation of antioxidant activity was done by DPPH (2, 2'-diphenyl-1-picrylhydrazyl) assay. 0.1mM DPPH solution was prepared in methanol (4mg/100ml). Different concentrations of test sample ranged between 10 to 100µg/ml were prepared in methanol. 2ml of test sample was added along with 1ml of DPPH solution. Incubation was given for 20 minutes. Absorbance was taken at 515 nm against blank (Methanol). Percentage inhibition was calculated using formula:

$$\% \text{ inhibition} = \frac{\text{A of Control} - \text{A of Sample}}{\text{A of Control}} \times 100$$

III. RESULTS AND DISCUSSION

The antioxidant activities were expressed in terms of % of inhibition and IC_{50} value. All absorbance values were taken in triplates. The % inhibition values of different concentrations in case of black grape methanolic extract ranged from 7.15 to 7.65 and its IC_{50} value came out to be 7.31µg/ml whereas the % inhibition values of different concentrations of green grape methanolic extract ranged from 5.50 to 8.07 % and its IC_{50} value came out to be 6.42µg/ml.

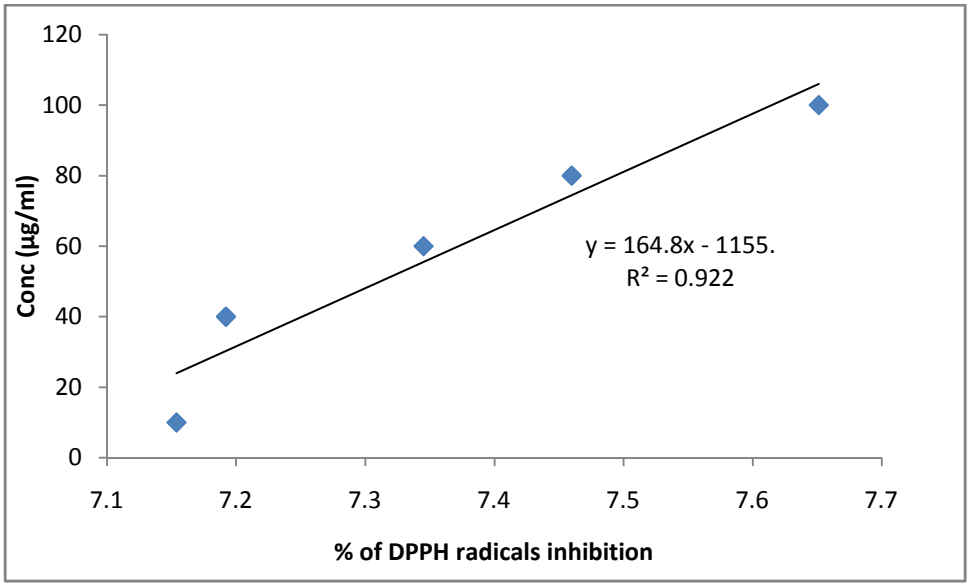

Fig. 1. Antioxidant (DPPH scavenging) activity of Black Grape methanolic extract presented as percentage of DPPH radicals inhibition.

The antioxidant activities of both green and black grapes were determined using a methanol solution of DPPH reagent. DPPH is very stable free radical. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecules quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless/bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band (1).

In a previous study, the IC_{50} for the Grape seed extracts (GSEs) of Italian Riesling and Župljanka grapes were 0.79 and 0.95 mg sample/mg DPPH radical, respectively, and it is in the same range as the values obtained by Bakkalbaşi *et al* [6] for the GSEs of the white grape varieties using acetone for the extraction (0.52–0.82 mg sample/mg DPPH radical).

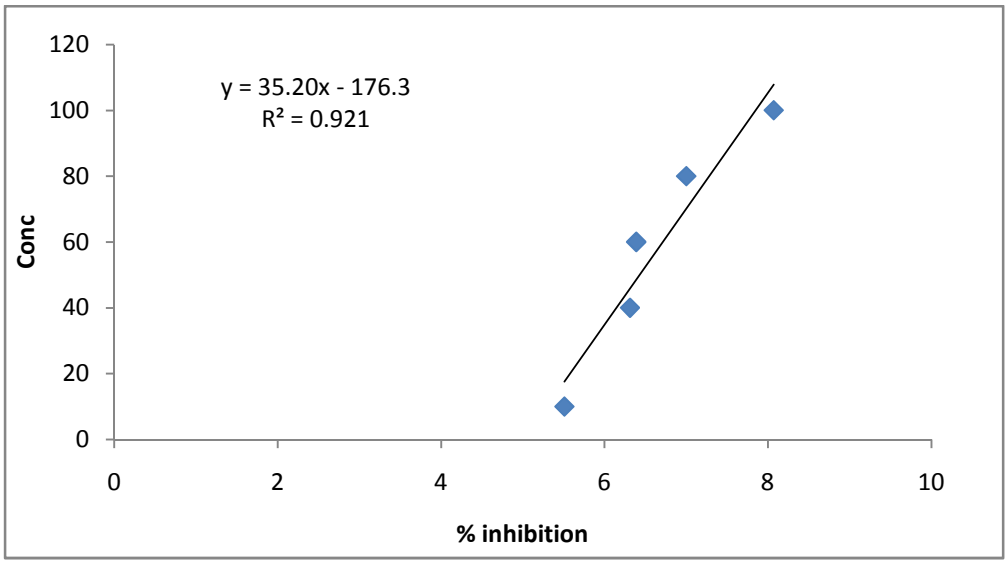

Fig. 2. Antioxidant (DPPH scavenging) activity of Green Grape methanolic extract presented as percentage of DPPH radicals inhibition.

Green grape methanolic extracts showed lower IC_{50} value i.e. 6.42 µg/ml as compared to the black grape methanolic extract which showed IC_{50} value 7.31µg/ml. This depicts that green grapes are potential antioxidants when compared against black grapes.

IV. CONCLUSION

Results of our study suggest the use of grapes in pharmacy and phytotherapy. Based on this information, it could be concluded that grapes are natural sources of antioxidant substances of high importance. The antioxidant activity of extracts obtained from green and black grapes showed that green grapes have greatest potential as a source of compounds to be applied as natural antioxidants in food. Since grape fruit is easily available so its use as an antioxidant will be a good drug of choice and poor men friendly.

REFERENCES

[1]. Amarowicz, R., Pegg, B.R., Rahimi-Moghaddam, P., Bar, B., Weil, J.A. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem*.2003, **84**, 551-562.

[2]. Arts, I.C.; Hollman, P.C. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr*.2005, **81,** 317S–325S.

[3]. Arvanitoyannis, I.S., D. Ladas and A. Mavromatis: Potential Uses and Applications of Treated Wine Waste: A Review. *Int. J. Food Sci. Tech*. 2006, **41,** 475-487.

[4]. Bagchi, D.; Bagchi, M.; Stohs, S.J.; Das, D.K.; Ray, S.D.; Kuszynski, C.A.; Joshi, S.S.; Pruess, H.G. Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. *Toxicology*. 2000, **148**, 187–197.

[5]. Bajaj, S., A. Urooj and P. Prabhasankar: Effect of Incorporation of Mint on Texture, Colour and Sensory Parameters of Biscuits. *Int. J. Food Prop*. 2006, **9,** 691-700.

[6]. Bakkalbasi, E., O.Yemi, D. Aslanova and N. Art: Major Flavan-3-ol Composition and Antioxidant Activity of Seeds from Different Grape Cultivars Grown in Turkey. *Eur. Food Res. Technol.* 2005, **221,** 792-797.

[7]. Bravo, L. Polyphenols: Chemistry, Dietary Sources, Metabolism and Nutritional Significance. *Nutr. Rev.* 1998, **56**, 317-333.

[8]. Cano, A. and M.B. Arnao: Hydrophilic and Lipophilic Antioxidant Activity in Different Leaves of Three Lettuce Varieties. *Int. J. Food Prop*. 2005, **8**, 521-528.

[9]. Castilla, P.; Echarri, R.; Dávalos, A.; Cerrato, F.; Ortega, H.; Teruel, J.L.; Lucas, M.F.; Gómez-Coronado, D.; Ortuño, J.; Lasunción, M.A. Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *Am. J. Clin. Nutr*. 2006, **84**, 252–262.

[10]. Dröge, W. Free radicals in the physiological control of cell function. *Physiol. Rev*. 2002, **82**, 47–95.

[11]. Halliwell, B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? *Lancet* 1994, **344**, 721–724.

[12]. L.S. Einbond, K.A. Reynertson, X.-D. Luo, M.J. Basile, E.J. Kennelly Anthocyanin antioxidants from edible fruits. *Food Chemistry*, 2004, **84**, 23–28.

[13]. Moure, A., J.M. Cruz, D. Franco, J.M. Domínguez, J. Sineiro, H. Domínguez, M.J. Núñez and J.C. Parajo. Natural Antioxidants from Residual Sources. *Food Chem*, 2001, **72**,145-171.

[14]. Pokorny, J., N.Yanishlieva, and M.Gordon. Introduction of Antioxidants in Food. *CRC Press and Woodhead Publishing Limited, Cambridge, England*, 2001, 1-3.

[15]. Robards, K. Strategies for the Determination of Bioactive Phenols in Plants, Fruit and Vegetables. *J. Chromatogr*. *A.* 2003, **1000**, 657-691.
[16]. Sano, A.; Uchida, R.; Saito, M.; Shioya, N.; Komori, Y.; Tho, Y.; Hashizume, N. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. *J. Nutr*. *Sci. Vitaminol*. 2007, **53**,174–182.
[17]. Shanmuganayagam, D.; Beahm, M.R.; Osman, H.E.; Krueger, C.G.; Reed, J.D.; Folts, J.D. Grape seed and grape skin extracts elicit a greater antiplatelet effect when used in combination than when used individually in dogs and humans. *J. Nutr*. 2002, **132**, 3592–3598.
[18]. S.E. Soares Ácidos fenólicos como antioxidants. *Revista de Nutrição*. 2002, **15**(1), 71–81.
[19]. Walzem, RL: Wine and health: state of proofs and research needs Inflammopharmacology. 2008, **16**, 265-271.
[20]. Waterhouse AL: Wine phenolics. *Annals of The New York Academy of Sciences.* 2002, **957**, 21-36.
[21]. Yildirim, H. K., Akçay, Y. D., Güvenç, U., Altindisli, A., & Sözmen, E. Y. Antioxidant activities of organic grape, pomace, juice, must, wine and their correlation with phenolic content. *International Journal of Food Science and Technology*. 2005, **40**, 133–142.
[22]. Zin, Z.M., A.A. Hamid, A. Osman, N. Saari and A. Misran: Isolation and Identification of Antioxidative Compound from Fruit of Mengkudu (*Morinda citrifolia* L.). *Int. J. Food Prop.* 2007, **10**, 363-373.