JSR Publications

Available Online

J. Sci. Res. **6** (1), 185-190 (2014)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Short Communication

Evaluation of Antiradical Activity Grape Seeds and Olive (*Olea europaea*) Pits Extracts

F. Tajalli*

Molecular Medicine Research Department, ACECR-Mashad Branch, Ferdowsi University, Azadi Square, Mashad, Khorasan Razavi, Iran

Received 26 October 2013, accepted in final revised form 16 December 2013

Abstract

The antiradical activity of grape seeds and olive pits extracts were investigated. The aim of this study is evaluating the radical scavenging activity of methanol extracts of olive pits and grape seeds and to recover a functional and antiradical compound from olive oil and fruit juices factories waste and also for environmental treatment. The antiradical properties of ripe and unripe olive pits (RIOP and URIOP) and grape seeds (IGS) that are respectively used in Iranian oil industries and fruit juices producers are examined. All seeds and pits extracts showed DPPH radical scavenging activity ranging from 24.51 to 97.06. For this purpose a methanolic extract was prepared from each of the RIOP, URIOP and IGS and their radical scavenging ability is determined with DPPH method. For this trial the effect of 3 different dilutions (100, 200 and 300 µg/L) of RIOP, URIOP, IGS extracts was used, separately. It was appeared that in above 100 ppm concentrations the antiradical properties reaches to its maximum activity. Also, IGS extract shows better effects in 100 ppm concentrations in comparison with RIOP, URIOP and ascorbic acid. The study shows that grape seeds can be used as a rich source of functional and antiradical compound and anticancer drugs production.

Keywords: Iranian olive pits; DPPH; Antiradical activity; Radical scavenging; Grape seeds**.**

doi: http://dx.doi.org/10.3329/jsr.v6i1.16654 J. Sci. Res. **6** (1), 185-190 (2014)

1. Introduction

A free radical has an unpaired electron that is produced by radiation or is a by-products of metabolic processes [1, 2]. They begin to disintegrate the cell membranes and cellular compounds via to damage biomembrane components as lipids, proteins and DNA in and decrease membrane fluidity [1, 3, 4]. Two species of these free radicals are reactive

* *Email:* tajalli@acecr.ac.ir

oxygen species (ROSs) like a superoxide anion (O_2·), hydroxyl (OH·), hydroperoxyl (OOH·), peroxyl (ROO·), alkoxyl (RO·), radicals non free radicals are hydrogen peroxide (H2O2), hypochlorous acid (HOCl), ozone (O3) singlet oxygen (1O2) and reactive nitrogen species (RNSs) similar to a nitric oxide (NO·), peroxynitrite (ONOO·) nitrogen dioxide (NO2) [2]. Antioxidants are agents and molecules that can reduce and limit oxidative damage to biological structures by scavenging the free radicals, neutralize the harmful free radicals before ROS and RNS can attack the cells and prevent damage living cells, proteins, enzymes, DNA, spoil foods, lipids, carbohydrates and also degrade materials such as rubber, gasoline and lubricating oil [2, 5 6]. They break off the chain reactions via the removal of free radical and inhibit other oxidation reactions [5]. Also, these are classified into two major classes enzymatic and non-enzymatic [2].

In recent years, many epidemiological data have shown that consumption of vegetables and fruit as natural antioxidants may delay protect the human body from free radicals, prevent oxidative stress and or even prevent the onset of cardiovascular disorders, certain types of cancer, and other chronic dysfunctions, amoebic dysentery, chest pain, constipation, diarrhea, leucoderma and strangury, cough, fever, asthma, neurodegenerative diseases, aging, inflammation, atherosclerosis, diabetes [1-3, 7-9], because there are phenolics that exist in all of plants, flavonoids, anthocyanins, tannins and carotenoids which have the ability to protect the body from oxidative stress caused by free radical [1, 2, 10]. Totally, plants and animals protect themselves by a complex system from multiple antioxidants, such as glutathione, tocopherols, carotenoids, ascorbic acid, flavonoids and tannins, and vitamin E along with some enzymes like catalase, superoxide dismutase and various peroxidases [2, 5]. Therefore, pharmacognosy is an important therapeutic method for various diseases, as herbalism and folk medicine, both ancient and modern, were already existed in this fold [10]. On the other hand, there are some synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylated hydroquinone and gallic acid esters which are used in food industries and other materials that prompt negative health effects [2, 5, 6]. So, it is necessary the synthetic antioxidants are replaced with natural antioxidants due to their potential health risks and toxicity [1]. Despite of many done researches for discovery of novel natural antioxidants, there is still a demand to find more information on the antioxidant potential of plant species [5, 6]. In food industries, the health positive effects of certain vegetable oils, such as olive oil, are due to their fatty acid composition, rich in monounsaturated fat [11], and also due to their antioxidants [12, 13]. Many articles have shown, by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, free radical scavenging activity of nut, olive oil and grape using different solvents such as ethyl acetate or a mixture hexane/ethyl acetate/methanol [12, 14, 15]. Free radical scavenging activity is usually measured with the aim of determining possible biological effects of a sample rich in antioxidants since the latter have been related to the prevention of several diseases [16].

The aim of this study was the evaluating the radical scavenging activity of methanol extracts of olive pits and grape seeds due to recover a natural source of functional and antioxidant from olive oil and fruit juices factories waste and also for environmental treatment.

2. Materials and Methods

Methanol from Merck Co and DPPH from Sigma-Alderich Co were supplied. After harvest, pits of undamaged and disease-free ripe and unripe Iranian olive pits (RIOP and URIOP) were manually separated from pulp. Iranian Grape seeds (IGS) was taken from a fruit juice factory. Olive pits and grape seeds were dried at 40°C for 18 hours. Dried olive pits and grape seeds were ground to fine powder with a grinder. Then these powders (100g from each one) were extracted with 1000 mL methanol at room temperature for overnight.

2.1. *Measurement of antiradical activity (A_{AR})*

The DPPH assay measures the ability of the antioxidants present in the sample to scavenge free radicals, an important aspect to consider when measuring the biological activity of these compounds. This experimental procedure was adapted from Wang et al. (1998). To an methanol solution of DPPH (final concentration 0.1 mM), test extracts at different concentrations (100, 200 and 300 ppm) were added. The reaction mixtures were shaken vigorously and then kept in the dark for 20 min. The absorbance of the resulting solutions was measured in 1 cm cuvettes, using a CECIL Series 2 UV/VIS spectrophotometer at 517 nm, against blank without DPPH. Decrease of DPPH solution absorbance indicates an increase of DPPH radical-scavenging activity. This activity is given and calculated by formula (1):

$$\text{Radical Scavenging Activity} = \frac{A_{517(0)} - A_{517(20)}}{A_{517(0)}} * 100 \quad (1)$$

The DPPH solution without sample solution was used as control. All tests were run in triplicate and averaged. Ascorbic acid was used as positive control.

2.2. *Statistical analysis*

Data were expressed as means ± S.E. Statistical analysis was performed by employing student's t-test Differences. A_{AR} among RIOP, URIOP and IGS were compared by were considered significant at $p \leq 0.05$ by plotting the percentage of Radical Scavenging Activity versus the concentrations.

3. Results and Discussion

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. Fig. 1 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of the routine and standards. Antiradical activity of extracts varied from 24.51% to 97.06% (Table 1).

Table 1. Total antiradical activity (A_{AR}) of the olive pits, grape and currant seeds extracts.

Treatment	Concentration (ppm)		
	100	200	300
RIOP	39.98 b	60.39 c	82.94 e
URIOP	24.51 a	56.27 c	73.53 d
IGS	93.86 f	-	-
Ascorbic acid	84.7 e	81.17 e	97.06 f

Values reported are means of triplicate determinations (n=3).

We used *V*c as standard radical scavengers and in Fig. 1, antiradical scavenging activity of RIOP and URIOP extracts were compared ascorbic acid. It was appeared that in 300 ppm concentrations the antiradical properties reaches to its maximum activity and there is a little difference between RIOP and URIOP extracts in radical scavenging activity. But, there are many differences between olive pits and grape seeds extracts in antiradical scavenging activity (Fig. 2). IGS extract show better effects in 100 ppm concentrations in comparison with RIOP and URIOP. Between extracts, the highest value was found for IGS (100 ppm) whereas URIOP (100 ppm) exhibited the weakest activity. The scavenging effect of extracts and standard on the DPPH radical increased in higher mounts, and at the concentration of 300 mg/mL, the resulting for RIOP and URIOP were 82.94% and 73.53%, respectively. But, IGP reached a high scavenging efficiency toward DPPH radicals in 100 mg/mL, while ascorbic acid exhibited higher activity in 300 mg/mL. These results indicated that grape seeds as a natural resource has a noticeable effect on scavenging free radicals. Free radical scavenging activity was also increased with an concentration increase. These data clearly indicate that grape seed is a powerful

free radical inhibitor or scavenger and can be used as a rich source of functional and antiradical compound and anti-cancer drugs production.

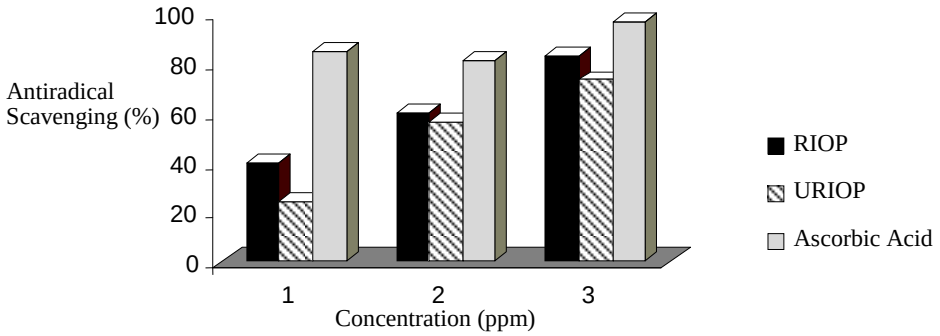

Fig. 1. Antiradical Scavenging Activity (A_{AR}) of Olive Pits and Ascorbic Acid.

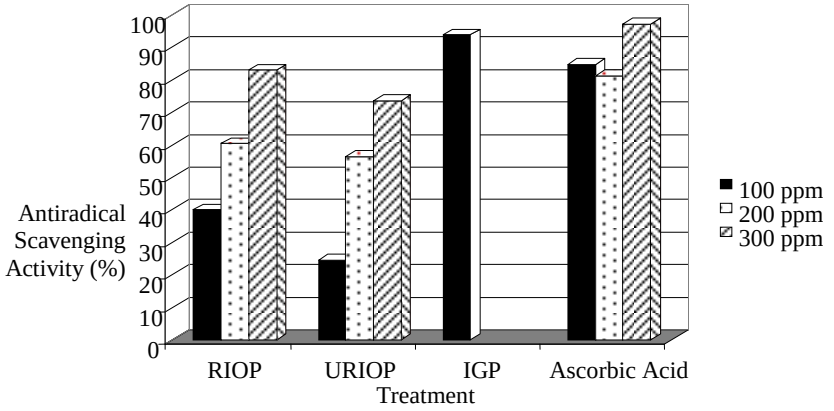

Fig. 2. A_{AR} of olive pits and grape seeds extracts compare with ascorbic acid.

Acknowledgment

I thank my superior at Food Science & Technology Research Institute-ACECR, Mashhad Branch for the support of this project. My thanks go to Amir Salari for technical assistance in the project.

References

1. A. Anjum, M. A. Sikder, M. R. Haque, C. M. Hasan, and M. A. Rashid, J. Sci. Res. 5, 2, 343 (2013). http://dx.doi.org/10.3329/jsr.v5i2.13568

2. S. Naskar, A. Islam, U. K. Mazumder, P. Saha, P. K. Haldar, and M. Gupta, J. Sci. Res. **2** (1), 144 (2010). DOI: 10.3329/jsr.v2i1.2643
3. D. Prajapati, N. Patel, K. Mruthunjaya, and R. Savadi, J. Sci. Res. **1** (3), 606 (2009). http://dx.doi.org/10.3329/jsr.v1i3.2266
4. M. R. Islam, M. S. Parvin, M. S. Islam, S. M. R. Hasan, and M. E. Islam, J. Sci. Res. **4** (1), 193-202 (2012). doi:10.3329/jsr.v4i.17148
5. T. Ahsan, M. A. Rahman, N. U. Ahmed, and S. B. Uddin, J. Sci. Res. **2** (3), 621 (2010). DOI: 10.3329/jsr.v2i3.4772
6. M. A. Rahman, M. S. Rana1, M. M. Zaman, S. A. Uddin, and R. Akter, J. Sci. Res. **2** (1) 169 (2010). DOI: 10.3329/jsr.v2i1.2872
7. J. W. Lampe, Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies, Am. J. Clin. Nutri. **70**, 475S (1999).
8. M. Aguilera, M. D. Mesa, M. C. Ramirez-Tortosa, J. L. Quiles, and A. Gil, Clin. Nutri. 22, 379 (2003). http://dx.doi.org/10.1016/S0261-5614(03)00038-4
9. S. Arranz, R. Cert, J. Pérez-Jiménez, A. Cert, and F. Saura Calixto, Food Chem. **110**, 985 (2008).
10. A. Chowdhury, T. K. Pal, M. A. Alam, M. S. Rahaman and M. A. Rashid, J. Sci. Res. **5** (1) 201 (2013). http://dx.doi.org/10.3329/jsr.v5i1.10955
11. J. Lopez-Miranda, J. Delgado-Lista, P. Perez-Martinez, Y. Jimenez-Gómez, F. Fuentes, J. Ruano, and C. Marin, Molec. Nutri. Food Res. **51** (10) 1249 (2007). PMid:17879993
12. M. Ramadan and J. Moersel, J. Food Compos. Anal. **19**, 838 (2006). http://dx.doi.org/10.1016/j.jfca.2006.02.013
13. M. Tripoli, M. Giammanco, G. Tabacchi, D. Di Majo, S. Giammanco, and M. La Guardia, Nutri. Res. Rev. **18**, 98 (2005). http://dx.doi.org/10.1079/NRR200495 PMid:19079898
14. J. C. Espin, C. Soler-Rivas, and H. J. Wichers, J. Agric. Food Chem. **48** (3), 648 (2000). http://dx.doi.org/10.1021/jf9908188 PMid:10725129
15. L. Li, R. Tsao, R. Yang, J. K. Kramer, and M. Hernandez, J. Agric. Food Chem. 55 (4), 1164 (2007).
16. S. A. Stanner, J. Hughes, C. N. Kelly, and J. Buttriss, Public Health Nutri. **7** (3), 407 (2004). http://dx.doi.org/10.1079/PHN2003543 PMid:15153272