International Journal of Plant Production 3 (4), October 2009
ISSN: 1735-6814 (Print), 1735-8043 (Online)
This is a refereed journal and all articles are professionally screened and reviewed.
SHORT COMMUNICATION

GUASNR
www.ijpp.info

In vitro antioxidant activity of berry (*Morus alba var.nigra*)

E. Nikkhah*, M. Khayami, R. Heidari

Department of Biology, Faculty of Science, Urmia University, Urmia, Iran.
*corresponding author; Email: tu8084@yahoo.com

Received 26 September 2008; Accepted after revision 10 August 2009; Published online 17 September 2009

Abstract

Antioxidant activity of Berry (Morus alba var. nigra) was investigated. For superoxide anion radical assay, the superoxide anion radicals were generated by a pyrogallol auto oxidation system, Nitric oxide radical inhibition by the use of Griess Illosvoy reaction and reducing power was determined according to the Oyaizu method. At least this sample was represented a potential antioxidant capacity that was increased with increase in concentration of extract.

Keywords: Anthocyanins; Antioxidant; Berry; ROS

Introduction

In the last few years, there has been a growing interest in providing natural antioxidants. The protective effects of fruits and vegetables against coronary heart disease, stroke, and cancer have been attributed to the presence of flavonoids and other phytochemicals (Cacace and Mazza, 2002). All aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages and repair enzymes to remove or repair damaged molecules. However, this natural antioxidant mechanism can be inefficient, and hence dietary intake of antioxidant compounds is important. Recent reports indicated that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human diseases (Odukoya et al., 2005). Many studies have demonstrated the antioxidant activities and health benefits of the anthocyanins occurring in various fruits and vegetables (Jiao et al., 2005). Anthocyanins which are colored substances found in plants are used in food, nutraceutical and pharmaceutical preparations for having most of the red, purple and blue colors which have high potential as colorants because of their low toxicity (Ersus and Yurdagel, 2007). Berries are rich in anthocyanins, compounds that provide pigmentation to fruits and serve as natural antioxidants. Earlier studies have shown that berry anthocyanins are beneficial in reducing age associated oxidative stress, as well as in improving neuronal and cognitive brain function (Baghchi et al., 2003). The objective of this study was to investigate the scavenging capacities towards superoxide anion radicals, and nitrite radicals and reducing power of the Berry's anthocyanin extract as a potential source of natural functional substances for use as dietary antioxidants.

Materials and Methods

The extraction of anthocyanins was done as described by Chiriboga and Francis (1970). The total content of monomeric anthocyanins in Berry's anthocyanin extract was determined using the pH-differential method (Giusti and Wrolstad, 2001). For superoxide anion radical assay, the anion radicals were generated by a pyrogallol autoxidation system (Jing and Zhao, 1995). Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction (Garrat, 1964). The reducing power of Berry was determined according to the Oyaizu method (Oyaizu, 1986). Statistical analysis of the data was performed by ANOVA using SAS.

Results and Discussion

As shown in Figure 1, the inhibition effect of Berry's anthocyanin extract on the autoxidation of pyrogallol were relatively feeble at lower concentrations, but it exhibited strong inhibition activities at higher concentrations. Superoxide anion is an initial free radical and plays an important role in the formation of other ROSs such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems (Stief, 2003). It can also react with nitric oxide and form peroxynitrite, which can generate toxic compounds such as hydroxyl radical and nitric dioxide (Halliwell, 1997). Pyrogallol can autoxidate fast in alkali conditions and release superoxide anions, and, in return, the superoxide anions can accelerate the autoxidation (Zhonggao et al., 2005). As mentioned above, the superoxide anions were generated by the oxidation of pyrogallol and the scavenging effects were expressed as the inhibition of pyrogallol autoxidation, so any substance existing in the reaction system that might have effects on the oxidation of pyrogallol might affect the test results. Since the anthocyanin pigments from Berry were a crude extract, there might be some substance that could enhance the oxidation of pyrogallol and thereby offset some inhibition effects. It was therefore suggested that anthocyanins could be beneficial in scavenging free radicals. The scavenging of nitric oxide by Berry's anthocyanin extract was increased in a dose-dependent manner as illustrated in Figure 2. Nitric oxide radical inhibition study proved that anthocyanin pigments of Berry are a potent scavenger of nitric oxide. The nitric oxide generated from sodium nitro prusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci et al., 1994). Tsuda T et al., (2000) showed that anthocyanins can function as a potent inhibitor of the formation of nitrated tyrosine in vitro, and clarified how Pelargonidin, which has one hydroxyl group on the B-ring, scavenges peroxynitrite by detection of the nitrated reaction products (4-hydroxy-3-nitrobenzoic acid). They also demonstrated that cyaniding-3-glucoside, which is one of the typical anthocyanins, has antioxidant activity and protective effects against hepatic ischemia-reperfusion injury in vivo. Pergola et al., (2006) reported nitric oxide biosynthesis inhibition by blackberry’s anthocyanin extract. Their studies reported that part of anti inflammatory activity of blackberry extract was related to nitric oxide production inhibition by cyaniding-3-glucoside that it is major anthocyanin in blackberry extract. Figure 3 shows the reductive capabilities of Berry's anthocyanin extract. The reducing power of Berry's anthocyanin extract was potent and the power of the extract was increased

with quantity of sample. For the measurements of the reductive ability, the transformation of Fe^{3+} to Fe^{2+} was investigated in Berry's anthocyanin extract as described by Oyaizu et al., (Oyaizu, 1986). The reducing power increased with increasing the amount of extract. Baea and Suh (2007) studied antioxidant activities of five different mulberry cultivars in Korea, and found out that the high value of reducing power was an indication that some compounds in mulberry extract were both electron donors which could react with free radicals and be converted into more stable products thereby terminating free radical chain reactions. Compelling evidence indicates that increased consumption of dietary antioxidants or fruits and vegetables with antioxidant properties may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative diseases associated with aging (Mukherjee, 2003; Rajan et al., 2002). Therefore, the anthocyanin pigment in berry is a natural, edible colorant with excellent antioxidant properties and health benefits and it seems applicable in both health food and medicine.

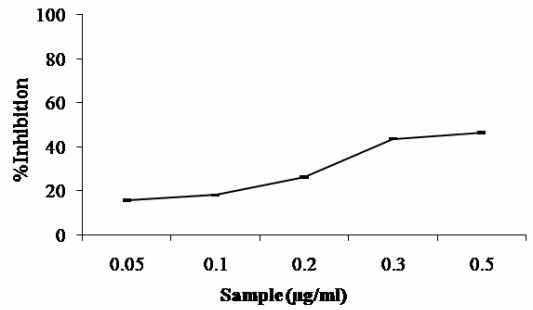

Figure 1. Scvenging capacity toward superoxide anion radicals by anthocyanin Extract from *Morus alba var nigra*. Mean of 3measurements ± SE.

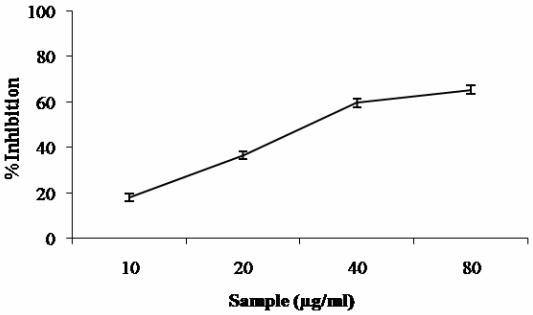

Figure 2. Nitric Oxide radical inhibition by anthocyanin Extract from *Morus alba var nigra*. Mean of 3measurements ± SE.

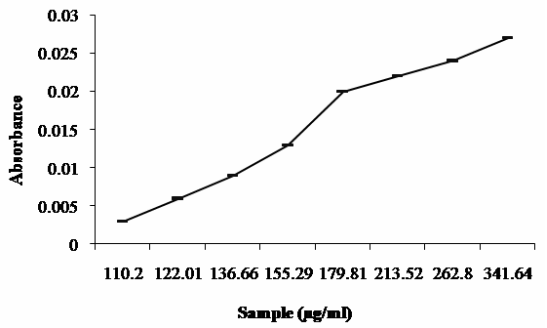

Figure 3. Reducing power of anthocyanin Extract from *Morus alba var nigra*. Mean of 3measurements ± SE.

References

Baea, S.H., Suh, H.J., 2007. Antioxidant activities of five different mulberry cultivars in Korea. *LWT*. 40: 955-962.

Baghchi, D., Sen, C.K., Baghchi, M., Atalay, M., 2003. Anti angiogenic, Antioxidant, and Anti carcinogenic Properties of a Novel Anthocyanin Rich Berry Extract Formula. Biokhimiya. 69: 1-8.

Cacace, J.E., Mazza, G., 2002. Extraction of Anthocyanins and Other Phenolics from Black Currants with Sulfured Water. J Agric Food Chem. 50: 5939-5946.

Chiriboga, C., Francis, F.J., 1970. An anthocyanin recovery system from cranberry pomace. J Am Soc Hort Sci. 9: 223-236.

Ersus, S., Yurdagel, U., 2007. Microencapsulation of anthocyanin pigments of black carrot (Daucuscarota L.) by spray drier. J Food Engin. 80: 805-812.

Garrat, D.C., 1964. The Quantitative analysis of Drugs. Chapman and Hall ltd. Japan. 3: 456-458.

Giusti, M.M., Wrolstad, R.E., 2001. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. In: Current Protocols in Food Analytical Chemistry, Wrolstad, R.E. (Ed.), John Wiley & Sons, New York, Pp: 1-13.

Halliwell, B., 1997. Antioxidants and human disease: A general introduction. Nutr Rev. 55: 44-49.

Jiao, Z., Liu, j., Wang, S., 2005. Antioxidant Activities of Total Pigment Extract from Blackberries. Food Technol. 43: 1. 97-102.

Jing, T.Y., Zhao, X.Y., 1995. The improved pyrogallol method by using terminating agent for superoxide dismutase measurement. Progr Biochem Biophys. 22: 84-86.

Marcocci, L., Packer, L., Droy-Lefai, M.T., Sekaki, A., Gardes-Albert, M., 1994. Antioxidant action of Ginkgo biloba extracts EGb 761. Methods Enzymol. 234: 462-475.

Mukherjee, P.K., 2003. GMP for Indian system of medicine. In: GMP for Botanicals Regulatory and quality issues on Phytomedicines. Mukherjee, P.K., Verpoorte, R. (Eds), Business Horizons, New Delhi India, Pp: 99-112.

Odukoya, A., Ilori, O., Sofidiya, M., Aniunoh, O., Lawal, B., Tade, I., 2005. Antioxidant activity of Nigerian dietary spices. EJEAF Che. 4: 6. 1086-1093.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 44: 307-315.

Pergola, C., Rossi, A., Dugo, P., Cuzzocrea, S., Sautebin, L., 2006. Inhibition of nitric oxide biosynthesis by anthocyanin fraction of blackberry extract. Nitric Oxide. 15: 1. 30-39.

Rajan, S., Seturaman, M., Mukherjee, P.K., 2002. Ethnobiology of the Nilgiri hills, India. Phytother Res. 16: 98-116.

Stief, TW., 2003. The physiology and pharmacology of singlet oxygen, Med Hypotheses. 60: 567-572.

Tsuda, T., Kato, Y., Osawa, T., 2000. Mechanism for the peroxynitrite scavenging activity by anthocyanins. Feder. Europ. Biochem. Soci. Let. 484: 207-210.

Zhonggao, J., Jiechao, L., Sixin, W., 2005. Antioxidant Activities of Total Pigment Extract from Blackberries. Food Technol. Biotechnol. 43: 1. 97-102.