Research Paper

Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

Determination of Antioxidant Activity of Citrus Species using Different Solvent Extracts by Hydrogen Peroxide Scavenging Method

Biotechnology

KEYWORDS : Citrus species, Antioxidants, Solvent extracts, Phytochemical compounds.

*** Prasad.M.P** — Sangenomics Research Labs, Domlur Layout, Bangalore-560071, India. *Corresponding Author

Ashwin Rajkumar.R — St.Michael College of Engineering & Technology, Kalayarkoil, Sivaganga (DT), Tamilnadu.

ABSTRACT

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition. In the present investigation, the methanolic extracts of the C. aurantifolia, C. hystrix, Murraya koenigii and C. medica exhibited the maximum activity 45.75%, 57.08%, 65.04%, 63.87% at 100 µg/ml respectively. The ethanolic extracts which showed the maximum activity in all the samples 57.73% (C. aurantifolia), 49.37% (C. hystrix), 65.18% (C. maxima), 57.98% (C. reticulata), 76.30% (Murraya koenigii) and 61.6% (C. medica) at 100 µg/ml concentration. The hexane extract of C. aurantifolia (73.6% - 79.79%), C. hystrix (6.97% - 75.72%) and Murraya koenigii (67.32% - 81.68%) which revealed the maximum scavenging activity and the C. maxima, which showed the activity in the range 30.81% - 84.22%.

INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons and involved in many pathological conditions (Madhavi et al., 1996). It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants (Stanner et al., 2000). Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic (Branen 1975). Plant polyphenolic compounds, such as flavonoids are described as scavengers of reactive oxygen species (Chen et al., 1993). Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated (Rice-Evans 1994). Most sources of natural antioxidants originate from plant materials, but the content of polyphenolic compounds in the roots and pericarp of tropical and sub-tropical flora have sparsely reported (Elizabeth M., and Williamson, 2002).

Reactive oxygen species (ROS) generated in cells, are fundamental in modulating various physiological functions and represent an essential part of aerobic life and metabolism. Excessive generation of these radicals disrupts the antioxidant defense system of the body which may lead to oxidative stress (Mandal et al., 2009). Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, aging, cardiovascular and neurodegenerative diseases (Ebrahimzadeh et al., 2010). The plant species have been investigated in the search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore in recent years, considerable attention has been directed towards the identification of plants with antioxidant activity (El-Hela A, 2010).

Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Fernandez-Lopez et al., 2005; Jayapraksha and Patil, 2007; Ebrahimzadeh et al., 2004). Flavanones, flavones and flavonols are three types of flavonoids which occur in citrus fruit (Calabro et al., 2004). The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin (Mouly et al., 1994; Schieber et al., 2001). Epidemiological studies on dietary citrus flavonoids improved a reduction in risk of coronary heart disease (Di Majo et al., 2005; Hertog et al., 1993) and are attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Stavric, 1993; Elangovan et al., 1994; Martin et al., 2002). The interest in these classes of compounds is due to their pharmacological activity as radical scavengers (Cotelle et al., 1996).

Hydrogen peroxide is a weak oxidizing agent and it is not very reactive, can cross biological membranes. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus removing H_2O_2 is very important for the protection of living systems (Van Wijk R, et al., 2008).

MATERIALS AND METHODS

Collection and processing of plant samples

The leaves of C. aurantifolia, C. hystrix, C. maxima, C. reticulata, Murraya koenigii and C. medica were obtained from University of Agricultural Sciences, GKVK, Bangalore. The plant samples were transported in polythene bags to the lab where the study was carried out.

The leaves of four different samples were taken in four different trays and were dried under shade at room temperature for 3 weeks. The dried plant samples (leaves) were taken separately and ground using an electric blender to obtain a fine powder. The powdered samples were stored in a clean glassware container until further use

Solvent extraction process

10 gs of the powdered plant samples [C. aurantifolia, C. hystrix, C. maxima, C. reticulata, Murraya koenigii and C. medica] was dissolved in 100 ml of solvent (methanol, hexane and ethanol) and incubated at room temperature for 48 h. The extracts were filtered through a Whatmann filter paper and concentrated using a rotary evaporator at 40 °C and stored for further use.

Assay

The ability of the extracts to scavenge hydrogen peroxide ($H_2O_{2)}$ was determined according to the method of Nabavi et al., 2008). Solution of hydrogen peroxide (40mM) was prepared in phosphate buffer, pH 7.4. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (100-1000 µg/ml) in solvents (methanol, ethanol and hexane) were added to hydrogen peroxide solution and the absorption measured at 230 nm after incubation for ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard.

RESULTS AND DISCUSSION

The Scavenging activity for methanolic extract ranged from 1.302% (Citrus reticulata) to 65.04% (Murraya koenigii) (Fig-

ure-1). The scavenging activity for ethanolic extract compareds with Ascorbic acid as standard ranged from 1.87% (Citrus medica) to 76.30% (Murraya koenigii) (Figure-2) and the Scavenging activity for hexane extract ranged from 1.51% (Citrus medica) to 84.22% (Citrus maxima) (Figure-3).

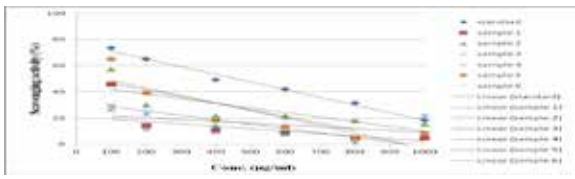

Figure-1: Scavenging activity of methanolic extracts of the Citrus sp.

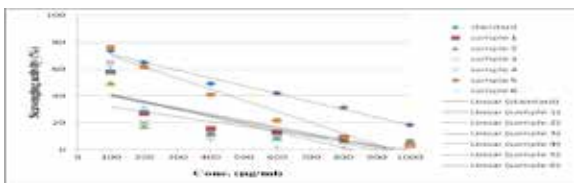

Figure-2: Scavenging activity of ethanolic extracts of the Citrus sp. which is compared with the L-Ascorbic acid (Standard).

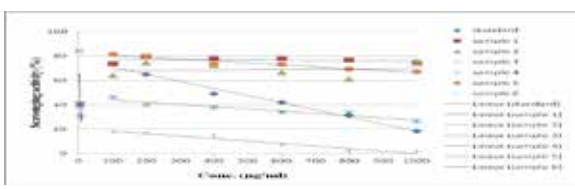

Figure-3: Scavenging activity of hexane extracts of the Citrus sp.

In the present study, the methanolic extracts of all the Citrus sp. showed consistent activity in all the concentrations. The methanolic extracts of the C. aurantifolia, C. hystrix, Murraya koenigii and C. medica exhibited the maximum activity 45.75%, 57.08%, 65.04%, 63.87% at 100 µg/ml respectively. The ethanolic extracts which showed the maximum activity in all the samples in the order 57.73% (C. aurantifolia), 49.37% (C. hystrix), 65.18% (C. maxima), 57.98% (C. reticulata), 76.30% (Murraya koenigii) and 61.6% (C. medica) at 100 µg/ml concentration. The hexane extract of C. aurantifolia (73.6% - 79.79%), C. hystrix (6.97% - 75.72%) and Murraya koenigii (67.32% - 81.68%) which revealed the maximum scavenging activity and the C. maxima, which showed the activity in the range 30.81% - 84.22%. C. reticulata, which also showed a consistent activity from 26.64% – 46.43%. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells (Halliwell, 1991).

CONCLUSION

The methanol, ethanol and hexane extracts of Citrus sp. showed hydrogen peroxide scavenging and powerful total antioxidant activities when compared to the standard (L- ascorbic acid). The results of this study suggests that all the three extracts of the six Citrus sp. possess high free radical scavenging activity which might be useful for further studies to unravel novel treatment strategies for diseases associated with free radical induced damage.

REFERENCE

1. Branen A.L (1975). J. American oil chemist's society, 52: 59-63. Chen S., Hwang J., Deng P.S.K (1993). Archives of biochemistry and biophysics, 303: 72-77. | 2. Calabro ML, Galtieri V, Cutroneo P, Tommasini S, Ficarra P and Ficarra R (2004). Study of the extraction procedure by experimental design and validation of a LC method for determination of flavonoids in Citrus bergamia juice. J Pharm and Biomed Anal., 35: 349-Dean SG and Svoboda KP (1989). Antimicrobial activity of summer savory (Satureja hortensis L.) essential oil and its constituents. J. Hortic. Sci. 64: 205-210 | 3. Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC and Gaydou EM (1996). Antioxidant properties of hydroxylflavones. Free Radic Biol Med., 20(1): 35-43. | 4. Di Majo D, Giammanco M, La Guardia M, Tripoli E, Giammanco S and Finotti E (2005). Flavanones in Citrus fruit: Structure-antioxidant activity relationships. Food Res. Intern., 38: 1161-1166. | 5. Ebrahimzadeh MA, Hosseinimehr SJ and Gayekhloo MR (2004). Measuring and comparison of vitamin C content in citrus fruits: introduction of native variety Chemistry: An Indian Journal, 1(9): 650-652. | 6. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of H. officinalis L. Var. Angustifoliu, V. odorata, B. hyrcana and C. speciosum. Pak J Pharm Sci 2010; 23:29-34. | 7. Elangovan V, Sekar N and Govindasamy S (1994). Chemoprotective potential of dietary bipoflavonoids against 20-methylcholanthrene- induced tumorigenesis. Cancer Lett. 87: 107-113. | 8. El-Hela A, Abudullah A. Antioxidant and antimicrobial activities of methanol extracts of some Verbena species: In vitro evaluation of antioxidant and antimicrobial activity in relation to polyphenolic content. J Appl Sci Res 2010; 6:683-689. | 9. Elizabeth M., Williamson (2002). Textbook of Major herbs of Ayurveda, (I): 7-11. | 10. Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez JA and Kuri V (2005). Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Sci., 69: 371-380 | 11. Halliwell, B. (1991) Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. American Journal of Medicine, 91, pp.14-22. | 12. Hertog MGL, Feskeens EJM, Holmann CH, Katan MB and Kromhout D (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. Lancet, 342: 1007-1011 | 13. Jayaprakasha GK and Patil BS (2007). In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange. Food Chem., 101: 410-418. | 14. Madhavi D.L., Deshpande S.S., Sulunkhe D.K (1996). Food antioxidants: technological, toxicological and health perspectives, New York, Marcel Dekker. | 15. Mandal S, Hazra B, Sarkar R, Biswas S, Mandal N. Assessment of the antioxidant and reactive oxygen species scavenging activity of methanolic extract of Caesalphinia crista leaf. Evid Based Complement Alternat Med 2009; 1-11. | 16. Martin FR, Frutos MJ, Perez-Alvarez JA, Martinez-Sanchez F and Del Rio JA (2002). Flavonoids as nutraceuticlas: structural related antioxidant properties and their role on ascorbic acid preservation. In: Atta-Ur-Rahman (editor), Studies in natural products chemistry Elsevier Science, Amsterdam, pp.324-389. | 17. Mouly PP, Arzouyan CR, Gaydou EM and Estienne JM (1994). Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavonone glycosides. J. Agric. Food Chem., 42: 70-79. | 18. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica mey. Pharmacology online 2008a; 2:560-67. | 19. Rice-Evans C., Miller N.J (1994). Methods in enzymology, 234: 279-293. | 20. Stanner S.A., Hughes J., Kelly C.N., Buttriss J.A (2000). Review of the epidemiological evidence for the 'antioxidant hypothesis, Public Health Nutrition, 7: 401-422. | 21. Stavric B (1993). Antimutagens and anticarcinogens in foods. Food Chem. Toxicol., 32: 79-90. | 22. Van Wijk R, Van Wijk EP, Wiegant FA, Ives J. Free radicals and low-level photon emission in human pathogenesis: State of the art. Indian J Exp Biol 2008; 46:273-309. |