International Journal of Advanced Biotechnology and Research(IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol5, Issue3, 2014, pp295-298
http://www.bipublication.com

Antioxidant Assay of Citrus Species Using Different Solvent Extracts by Reducing Power Method

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[Received-21/04/2014, Accepted-02/07/2014]

ABSTRACT

Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease and in the aging process. The transformation of Fe3+ into Fe2+ in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom. The antioxidant principles present in the extracts caused the reduction of Fe3+/ ferricyanide complex to the ferrous form, and thus proved the reducing power ability. In the present investigation, the methanol extract exhibited good scavenging activity in all the samples. The activity was ranging from 92.57% (C. aurantifolia) to 14.63% (C. medica) in the methanol extract of samples. The hexane extract also showed similar activity in all the samples, in which the activity ranged from 90.33% (C. medica) to 19.52 (C. aurantifolia). In the ethanol extract, the activity ranged from 96.05% (C. maxima) to 22.53% (C. reticulata).

Keywords: *C. aurantifolia, C. hystrix, C. maxima, C. reticulata, Murraya koenigii and C. medica.* Phytochemicals.

INTRODUCTION

Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase.

Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns [1]. These factors have inspired the widespread screening of

plants for possible medicinal, antimicrobial and antioxidant properties [2]. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease and in the aging process [3]. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids.

Free radicals may cause reversible or irreversible damages to biological molecules such as DNA, proteins and/or lipids [4, 5]. These damages may cause cancer, heart diseases, arthrosclerosis, hypertension, arthritis, ischemia/reperfusion injury, and diabetes mellitus, neurodegenerative diseases (Alzheimer's disease and Parkinson's disease) and could accelerate aging of organisms [6].

Free radicals are types of reactive oxygen species (ROS), which include all highly reactive, oxygen containing molecules. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. These free radicals may either be produced by physiological or biochemical processes or by pollution and other endogeneous sources. All these free radicals are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage [7].

MATERIALS and METHODS

Collection and processing of plant samples:

The leaves of *Citrus aurantifolia, C. hystrix, C. maxima, C. reticulata, Murraya koenigii and C. medica* were obtained from **University of Agricultural Sciences, Bangalore**. The plant samples were transported in polythene bags to the Lab and the study carried out. The leaves of four different samples were taken in different trays and the leaves were dried under shade at room temperature for 3 weeks. The dried plant samples (leaves) were taken separately and grounded using an electric blender to obtain a fine powder and were stored in a clean glass containers until further use

Solvent extraction process:

1 g of the powdered plant samples [*C. aurantifolia, C. hystrix, C. maxima, C. reticulata, Murraya koenigii and C. medica*] were dissolved in 100 ml of methanol and extracted at room temperature for 48 h. The extracts were filtered through a Whatmann filter paper and concentrated using a rotary evaporator at 40 °C. Similar methodology was followed for the Hexane and Ethanol solvents.

Reducing power assay:

The assay was followed as per standard protocol [8]. The solvent extracts of the sample were taken in the following concentration range (100, 200, 400, 600, 800, 1000µl) in each test tubes and makeup with the solvent up to 1ml. 0.5 ml of 0.2 M Phosphate buffer (pH 6.6) and 0.5ml of (1%) Potassium ferrocyanide was added. After incubating the mixture at 50ºC for 20 minutes, 0.5ml of Trichloro acetic acid was added and then the mixture was centrifuged at 3000 rpm for 10 min. 1ml of supernatant was mixed with 1ml of distilled water and 0.2ml of (0.1%) $Fecl_3$ and the Absorbance was measured at 700nm in spectrophotometer. The experiments were performed in triplicate and the mean value taken.

Scavenging activity was calculated from control sample OD using the following equation

Radical scavenging activity (%) = [(A control –A test)/A control] x 100

RESULTS

The Reducing power scavenging assay was done for all the three solvent extracts of Ethanol, Methanol and Hexane. The results varied with each solvent extract and also the citrus species taken. The results are represented in the graphical form in Figure-1-3. From the present study, it is observed that the methanol extract of *C. aurantifolia* (92.57%) exhibited maximum reducing power activity wherein the minimum activity observed in *C. medica* (14.63%). Similarly in the hexane extract, the highest reducing power activity was observed in *C. maxima* (91.92%) and the minimal activity was exhibited by *C. aurantifolia* (19.52%). And the ethanol extract, revealed maximum reducing power

activity in *C. maxima* (96.05%) and minimum activity in *C. reticulata* (22.53%).

Methanolic extract

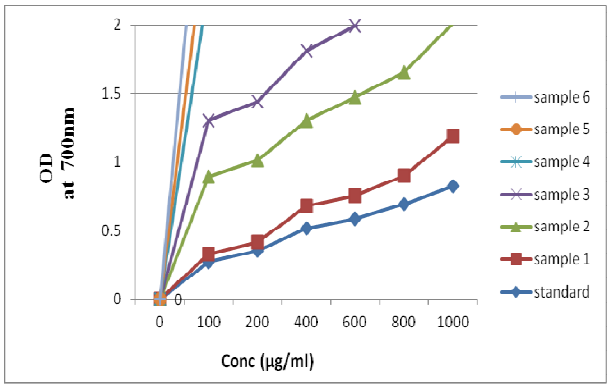

Figure-1: Reducing Power Scavenging Assay for methanolic extract

Hexane extract

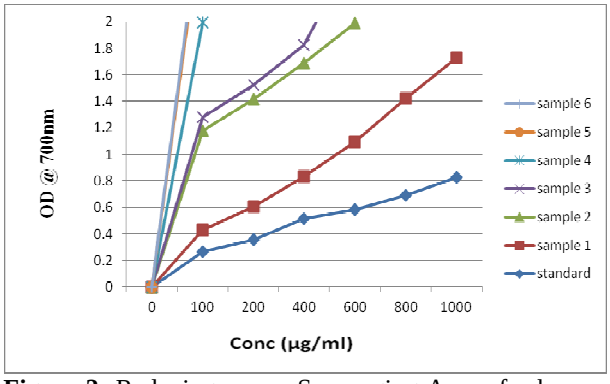

Figure-2: Reducing power Scavenging Assay for hexane extract

Ethanolic extract

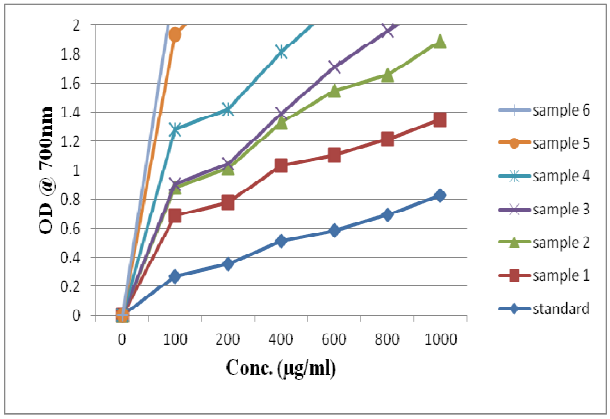

Figure-3: Reducing power Scavenging Assay for ethanol extract

DISCUSSION

The transformation of Fe3+ into Fe2+ in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom [9]. The antioxidant principles present in the extracts caused the reduction of Fe3+/ ferricyanide complex to the ferrous form, and thus proved the reducing power ability. From the results obtained, the methanol extract exhibited good scavenging activity in all the samples. The activity was ranging from 92.57% (C. aurantifolia) to 14.63% (C. medica) in the methanol extract of samples. The hexane extract also showed similar activity in all the samples, in which the activity ranged from 90.33% (C. medica) to 19.52 (C. aurantifolia). In the ethanol extract, the activity ranged from 96.05% (C. maxima) to 22.53% (C. reticulata).

CONCLUSION

Higher absorbance of the reaction mixture indicates higher reductive potential. The reducing capacity of the compound may serve as a significant indicator of its potential antioxidant activity. Further studies will help in identifying the synergistic effect. Also a correlation between the reducing power and antioxidant activity can be derived. In the present investigation, we have warranted the concentration dependent reducing ability of the extracts of Citrus sp.

REFERENCES

1. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr J Biotechnol. 2007; 6:1197-1201.
2. Aruoma IO. Free radicals, oxidative stress and antioxidants in human health and disease. American Oil Chemists Soc. 1998; 75:199–212.
3. Elmastasa M, Gulcinb I, Isildaka O, Kufrevioglub OI, Ibaoglua K, Aboul-Eneinc HY. Radical scavenging activity and antioxidant capacity of Bay Leaf Extracts. *J. Iran. Chem. Soc.* 3, 2006, 258-266.

4. Halliwell B and Gutteridge JMC. Free radicals in Biology and Medicine, 3rd ed. Oxford University Press, Oxford, pp. 1999, 23-27.
5. Jayaprakasha GK, Singh RP, Sakariah KK). Antioxidant activity of grape seed (*Vitrus vinifera*) extracts on peroxidation models *in vitro*. Food Chem. 2001; 73:285-290.
6. Meir S, Kanner J, Akiri B, Hadar SP. Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves. *J. Agric. Food Chem.* 43, 1995, 1813-1817
7. P.Jayanthi, P. Lalitha, International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 3, Suppl 3, 2011.
8. Shivaprasad HN, Mohan S, Kharya MD, Shiradkar R M, Lakshman K. In-vitro models for antioxidant activ ity evaluation: A review. Latest reviews 2005; 3(4).
9. Siger A, Nogala-Kalucka M, Lampart-Szczapa E. The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. *J. Food Lipids*. 15, 2008, 137–149.