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Antioxidant Activity of Bitter Orange (*Citrus aurantium* L.) Peel Powder

KEYWORDS: antioxidants, fruit peel powder, free radicals

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ABSTRACT *Recent studies have proved that citrus fruits apart from contributing vitamin C to the diet are also an abundant source of bioactive components such as phytonutrients and antioxidants. And these bioactive components which are potential therapeutic agents for degenerative diseases are present in their peels. The present study was aimed at determining the antioxidant activity of Bitter orange (Citrus aurantium L.) peel powder using three different methods (DPPH assay, FRAP assay and Hydrogen peroxide scavenging assay). From the results obtained from DPPH assay of Citrus aurantium L. peel powder using five different extracts (aqueous, acetone, ethanol, petroleum ether and chloroform), it was found that acetone had greater scavenging activity followed by ethanol, aqueous, and chloroform at 30 minutes. The antioxidant activity of Citrus aurantium L. peel powder using ethanol extract by FRAP assay was 2.010mM Fe/g indicating high antioxidant activity. The results for Hydrogen peroxide scavenging assay indicate that the percentage of scavenging activity by the standard Gallic acid was 57.32% and the test sample (Citrus aurantium L. peel powder ethanol extract) yielded 42.13% of scavenging activity.*

INTRODUCTION

Citrus fruits belong to the family rutaceae, are one of the main trees grown throughout the world (Okwu, 2006). Fruits are an important source of compounds called phytonutrients such as ascorbic acid, flavonoids, carotenoids, phenolic acid, tocopherol and sulphur containing compounds, which possess antioxidant properties (Paul et al., 2001). The bitter orange (*Citrus aurantium* L.) fruit is spherical, medium to dark-green, becoming more yellow as it ripens, and yields bitter orange oil. Its flowers and fruits are used in oriental medicine – mainly as remedies for myriad disorders of the digestive system, as a cardiac tonic and for anxiety (Rhind, 2014)

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Most of the phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer (Joshi, 2010). Antioxidants are secondary metabolites found naturally in plants such as citrus fruits. An antioxidant can be defined as anything that inhibits or prevents oxidation of a substrate. 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. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signalling and immune function (Gulcin, 2005). Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases (Hsu et al., 2003). Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation (Erkan et al., 2008). Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga et al., 2005; Mann,1978).

Figure 1: Bitter orange fruit

MATERIALS AND METHODS

The Bitter orange fruits were procured from the local market. The peels were separated from the fruit by hand in a sterilized condition. The peels were shade dried and then ground well to form a powder. The powdered bitter orange peel was stored in an airtight container. The peel extracts were prepared using the method described by Janarthanam et al., (2013). To each 20 ml of aqueous, ethanol, acetone, chloroform and petroleum ether 1 gram of dried bitter orange peel powder was added and left it

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overnight soaking at room temperature. The sample was then filtered through Whatman.No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extract was approximately 100%. The extracts were used for further tests.

2.1 Antioxidant activity of Bitter orange peel powder
The antioxidant activity of the different solvent extracts of peel powder was evaluated by DPPH free radical and it was further evaluated by other antioxidant parameters like FRAP assay and Hydrogen peroxide Scavening Assay.

2.1.1. Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity
Qualitative DPPH analysis

- 50µl of extracts were taken in the microtiter plate.
- 100µl of 0.1 methanolic 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added over the samples and incubated for 30 minutes, in dark condition.
- The samples were then observed for discolouration from purple to yellow and pale pink, these were considered to be strong and weak positive respectively.

Quantitative DPPH analysis
The antioxidant activities were determined using DPPH as a free radical. Extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially absorption of blank containing the same amount of methanol and DPPH solution was prepared and measured as a control. Subsequently at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan at 517nm. Antioxidant activity was compared with known synthetic standard of (0.16%) of BHT.

The capacity of scavenging free radicals was calculated as scavenging activity (%)

$$\text{Inhibition\%} = \frac{\text{(Absorbance of Control – Absorbance of Sample) x 100}}{\text{(Absorbance of control)}}$$

Ferric reducing antioxidant power (FRAP)
The reducing ability of different solvent extracts and fractions of the peel extract was determined by FRAP assay (Benzie and Strain, 1996). FRAP assay is based on the ability of antioxidants to reduce Fe3+ to Fe2+ in the presence of TPTZ, forming an intense blue Fe2+-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). 0.1 ml extract is added to 3.0 ml FRAP reagent [10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2,4,6- tripyridyl-s-triazine) in 40 mM HCl and 1 part 20 mM FeCl3] and the reaction mixture is incubated at 37°C for 10 min and then the absorbance was measured at 593 nm. FeSO4 (100 to 1000 µM ml-1) was used as a positive control (Thondre et al., 2011). The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as M FeSO4 equivalents per gram of extracted compound.

Hydrogen peroxide Scavenging Assay (H_2O_2 assay)
The scavenging activity of extract towards hydrogen peroxide radicals was determined by the modified method of Dehpour. Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560nm using UV spectrophotometer. 0.1mg/ml of the extract was added to hydrogen peroxide solution and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and stand compound was calculated using the given formula:

Percentage scavenged (H_2O_2) = 1 – Abs standard / Abs (Control) x 100

Where, Abs (control) was the absorbance of the control (without extract) at 560nm; Abs sample was the absorbance in the presence of the extract at 560nm.

3. RESULTS AND DISCUSSION
Antioxidant activity of Bitter orange peel powder
3.1.1. Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity
Qualitative DPPH analysis
The results of the present study as prescribed in Table 1, taken to change colour from purple to yellow was fastest in ethanol and acetone extract because of its highest antioxidant activity. This was followed by aqueous and chloroform, while petroleum ether extract did not show presence of antioxidant activity.

Table 1: Results for qualitative antioxidant activity of *Citrus aurantium* L. peel powder using DPPH analysis

Solvent Extract	Presence of Antioxidants
Aqueous	+
Ethanol	++
Acetone	++
Petroleum Ether	-
Chloroform	+
(++) Strong positive; (+) Weak positive ; (-) Absent	

Quantitative DPPH analysis
The decrease in absorbance of DPPH radical is caused by antioxidant through the reaction between antioxidant molecule and radical results in the scavenging of the radical by hydrogen donation (Hong, 1997). From the result obtained from the present study, it was found that the scavenging activity *Citrus aurantium* L. peel powder acetone extract had greater scavenging activity of 81.81% followed by ethanol with 78.51%, aqueous with 76.03% and chloroform with 57.85% at 30 minutes. The scavenging activity of *Citrus aurantium* L. peel powder chloroform extract remained constant with 49.58% from 0-30minutes (Figure 4)

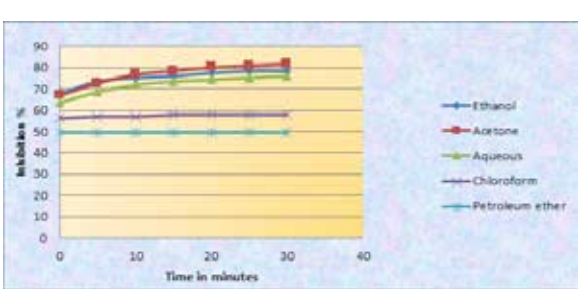

Figure 2: Antioxidant scavenging activity of *Citrus aurantium* L. peel powder by DPPH

Ferric reducing antioxidant power (FRAP)
The extract used for the FRAP assay to study the antioxi-

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dant power is ethanol as presented in Table 2.

Table 2: Results for FRAP assay of Citrus aurantium L. peel powder using ethanol extract

Sample	Absorbance	Concentration (Gram Per m Feso4 Equivalent)
Ethanol	0.60	2.010mM Fe/g

From the absorbance rate, the concentration of FeSo4 for ethanol extract is calculated. The result of the present study, the antioxidant power is 2.010mM Fe/g using ethanol extract indicating high antioxidant activity.

Hydrogen peroxide Scavenging Assay (H_2O_2 assay)

The principle of this method is that there is a decrease in absorbance of Hydrogen peroxide upon oxidation. Hydrogen peroxide can arise normally or sometimes the immune cells create them purposefully to neutralize the foreign bodies. Gallic acid is a positive control which is commercially available as standard. From Table 3, the result of Hydrogen peroxide indicates that the percentage of scavenging activity by the standard Gallic acid was 57.32% and the test sample (*Citrus aurantium* L. peel powder ethanol extract) yields 42.13% of scavenging activity.

Table 3: Hydrogen peroxide assay for *Citrus aurantium* L. peel powder

Sample	Percentage of scavenging activity
Standard (Gallic acid)	57.32%
Test sample	42.13%

Various researches have shown that some *C. aurantium* L. essential oils (Majnooni et al., 2012, Siddique et al., 2011) and extracts (Karimi et al., 2012, Muthiah et al., 2012 have free radical scavenging capacity. Limonene (87.02%), linalyl acetate (53.76%) and linalool (39.74%) were the major compounds of the essential oil extracted from peels, leaves and flowers of Tunisian *Citrus aurantium*. Tests on animals have proven the effectiveness of limonene against some types of cancer including gastric, mammary, pulmonary adenoma, and liver (Pasquale et al., 2006). Besides the effectiveness of limonene in traditional medicine to treat severe dermatitis, fatigue recovery (Parizzi et al., 1993), and depression (Fayed, 2009), different studies have reported its anxiolytic and antidepressant effects on the central nervous system (Yoko et al., 2008).

4. CONCLUSION

The present study was conducted to determine the antioxidant activity of Bitter orange (*Citrus aurantium* L.) peel powder. As synthetic antioxidants have side effects, search for natural source of antioxidants has gained attention recently. The study has proved that *Citrus aurantium* L. peel powder is rich in antioxidants. Therefore it has the potential to be used as an alternate for synthetic antioxidants.

5. REFERENCES

1. Ao. Yoko, K. Satoh, K. Shibano, Y. Kawahito, S. Shioda. (2008). Singlet Oxygen Scavenging Activity and Cytotoxicity of Essential Oils from Rutaceae, Journal of Clinical biochemistry and Nutrition, 43, 6-12.
2. E. Karimi , E. Oskoueian, R. Hendra , A. Oskoueian ,H. Z. E. Jaafar. (2012). Phenolic Compounds Characterization and Biological Activities of Citrus aurantium Bloom, Molecules, 17, 1203-1218.
3. Edoga HO, Okwu DE, Mbaebie BO (2005). Phytochemicals constituents of some Nigerian medicinal plants. Afr J Biotechnol; 4(7) : 685-688.
4. Erkan N, Ayranci G, Ayranci E. (2008). Antioxidant activity of rosemary (*Rosmarinus officinalis*) extract, Black seed (*Nigella sativa*) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chemistry; 110:76-82.
5. F. De Pasquale, M., Siragusa, L. Abbate, N. Tusa, C. De Pasquale, G. Alonzo. (2006). Characterization of five sour orange clones through molecular markers and leaf essential oils analysis, Scientia Horticulturae, 109, 54–59.
6. Gulcin I. (2005). The antioxidant and radical scavenging activities of black pepper seeds, International Journal of Food Science and Nutrition; 56:491-499.
7. Hill,A.F. (1979). Economic Botany a Textbook of Useful Plants and Plant Products, Tata McGraw Hill Publishing Company Ltd., New Delhi. pp. 409-410
8. Hong, C.J. and H.Chi- Tang. (1997). AntioxidantActivities of Orange Peel Extracts. J. Agric. Food Chem., 45: 2357.
9. Hsu C, Chen W, Weng Y, Tseng C. (2003). Chemical composition, physical properties, and antioxidant activities of yam fluors as affected by different drying methods. Food Chemistry; 83:85-92.
10. Janarthanam B & Sumathi E, 2010. Antimicrobial activity of Gymnema sylvestre leaf and callus extracts. Journal of Tropical Medicinal Plants, 11(2):143-147.
11. Joshi,S.A. (2010). Nutrition and Dietetics with Indian Case Studies, Tata McGraw Hill Education Private Limited, New Delhi. pp.135
12. L .Panizzi, G . Flamini, P.L. Cioni, I. Morelli. (1993). Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae, Journal Ethnopharmacology, 39, 167–170.
13. M.B. Majnooni, K. Mansouri , M. Bagher Gholivand, A. Mostafaie, Hamid-Reza Mohammadi-Motlagh, Nazanin-Sadat Afnanzade, Mir-Mehdi Abolghasemi and Marzieh Piriyaei. (2012). Chemical composition, cytotoxicity and antioxidant activities of the essential oil from the leaves of Citrus aurantium L. , African Journal of Biotechnology. 11(2), 498-503.
14. Manay,N.S and Shadaksharaswamy,M (2008) Food Facts and Principles, New Age International, New Delhi. pp. 150
15. Mann J. (1978) Secondary Metabolism. Oxford University press, London .Pp. 154.
16. Okwu, D.E and Emenike,I.N (2006) Evaluation of phytonutrients and vitamin content of citrus fruit, International Journal of Molecular Medicine and Advance Sciences.pp. 1-6
17. P.L. Muthiah , M. Umamaheswari, K. Asokkumar. (2012) In vitro antioxidant activities of leaves, fruits and peel extracts of citrus, International Journal of Phytopharmacy, 2, 13-20.
18. Paul,T, Giovannucci, E., Michels, K.B., Bergkvist, L., Hansen, H., Holmberg, L., Wolk, A. (2001). Fruit, Vegetables, Dietary Fiber, and Risk of Colorectal Cancer, JNCI J Natl Cancer Inst, 93 (7): 525-533
19. Rhind,J.P. (2014). Fragrance and Wellbeing: Plant Aromatics and Their Influence on the Psche, Singing Dragon Publications, Philadelpia. pp. 331, 332, 338
20. S. Fayed, (2009). Antioxidant and Anticancer Activities of Citrus reticulate (Petitgrain Mandarin) and Pelargonium graveolens (Geranium) Essential Oils , Research Journal of Agriculture and Biological Sciences, 5, 740-747.
21. S. Siddique, M. Shafique, Z. Parveen, S. J. Khan, R. Khanum. (2011). Volatile Components, antioxidant and antimicrobial activity of Citrus Aurantium Var. Bitter Orange Peel Oil, Pharmacologyonline, 2, 499-507.