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Antioxidant Activities of Orange Peel Extracts

A.E. Hegazy and M.I. Ibrahium

Department of Food Science and Technology,
Faculty of Agriculture, AL-Azhar University, Cairo, Egypt

Abstract: This work aimed to evaluate the efficiency of different organic solvents such as, methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate for extraction of (flavonoids and polyphenolic compounds(TFC and TPC respectively) from the orange peel. Also, the effect of these solvents on the yield percentage, chelating activity, antioxidant/radical scavenging capacity and reducing power ability of the produced extracts were investigated. The results revealed that all extracts of the orange peel exhibited variable antioxidant activity. Specially, the ethanolic extract showed the highest ($p < 0.05$) values for yield (%), TPC, TFC, chelating and antioxidant activities (% DPPH scavenging activity). It is concluded that, the solvent play a vital role in the extraction of the plant constituents. Specially, methanol and ethanol are high polar among the solvents used.

Key words: Orange Peel · Antioxidant Activity · Phenolic Contents · Flavonoids

INTRODUCTION

Orange constitutes about 60% of the total citrus world production. In 2008, 3.23 million tons of citrus fruit were produced in Egypt, contained 2.14 million tons of orange. A large portion of this production is addressed to the industrial extraction of citrus juice which leads to huge amounts of residues, including peel and segment membranes. Peels represent between 50 to 65% of total weight of the fruits and remain as the primary byproduct. If not processed further, it becomes west produce odor, soil pollution, harborage for insects and can give rise to serious environmental pollution [1]. In Egypt and in many Mediterranean countries, major quantities of the peel are not further processed. Some attempts were made to use these residues as livestock feed, although their low nutritional value [2].

The antioxidant property of the plant material is due to the presence of many active phytochemicals including vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, lignin, saponin, plant sterol …etc [3]. 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 [4-6].

Flavanones, flavones and flavonols are three types of flavonoids that occur in Citrus fruit [7]. The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin [8]. Epidemiological studies on dietary citrus flavonoids reduce the risk of coronary heart disease [9, 10]. Also, it is attracting more attention as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects [11, 12]. The interest in these classes of compounds is due to their pharmacological activity as radical scavengers [13].

Many studies have reported antioxidant and antibacterial effect of juice and edible parts of oranges of different origin and from different varieties [14-16]. As far as the peel is concerned, extracts from this part of the fruit were found to have a good total radical antioxidative potential [17, 18].

There is paucity of information regarding the inhibitory effects of orange peel extract on lipid oxidation. Therefore, the purpose of the present study was to evaluate the effect of using some different solvents (such as methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate) on the extraction efficiency of effective compounds (such as polyphenolic and flavonoid compounds) from the orange peel.

Corresponding Author: M.I. Ibrahium, Department of Food Science and Technology, Faculty of Agriculture, AL-Azhar University, Cairo, Egypt.

In addition, their effect on the yield percentage, chelating activity, antioxidant/radical scavenging capacity and reducing power ability of the produced extracts was investigated.

MATERIALS AND METHODS

Materials: Ripened and freshly harvested oranges (Baladi) fruits (season October 2010) were obtained from local market. Folin-Ciocalteu reagent, methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate were purchased from E. Merck. Ferrous sulphate, disodium ethylene diamine tetraacetate (Na2 EDTA), butylated hydroxyanisole, quercetin, gallic acid, 2,2-bipyridyl, HCl and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma Chemical Co. (St. Louis, Mo).

Methods

Preparation of Orange Peel Extracts (OPE): Orange fruits were washed by distilled water then peeled and their edible portions were carefully separated. The peels were air dried in a ventilated oven at 40°C for 48 h and ground to a fine powder and passed through a 24-mesh sieve according to the method described by Van-Acker *et al.* [19]. 100g powdered sample was extracted with either 800ml ethanol or methanol or dichloromethane or acetone or hexane or ethyl acetate at room temperature by Soxhelt extraction method for 6 h. The mixture filtered through a Whatman No. 2 filter paper for removal of peel particles. The residue was re-extracted twice under the same condition to ensure complete extraction. The extracts were filtered and evaporated to dryness under reduced pressure at 60°C by a rotary evaporator. The extracts were placed in dark bottles and stored in refrigerator at 4°C until use.

Yield Estimation: Yield was estimated according to the method described by Prashani *et al.* [20]. Each 10 ml extract was measured into a pre-weighed aluminum dish. The samples were kept in an oven at 85°C for 24 h, afterwards in desiccator for 12 h. The weight difference was used to calculate percentage yield as well as expressed in mg/10 ml.

Determination of Total Polyphenols Content: The total polyphenols were determined colorimetrically using Folin-Ciocalteu reagent according to the method described by Ebrahimzadeh *et al.* [5]. The extract samples (0.5 ml different dilutions) were mixed with Folin-Ciocalteu reagent (5 ml with distilled water by rate 1:10) for 5 min and 4 ml aqueous Na2CO3 (1 M) were added. The mixture was stand for 15 min and the polyphenols were determined by an automated UV-VIS spectrophotometer at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200 and 250 mg ml-1 solutions of gallic acid in methanol: water (50:50 v/v).

Determination of Total Flavonoids Content: Colorimetric aluminum chloride method was used for flavonoids determination according to the methods described by Calabro *et al.* [7] and Ebrahimzadeh *et al.* [21]. 0.5 ml solution of each plant extracts was separately mixed with 1.5 ml methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible Spectrophotometer. Total flavonoid contents were calculated as quercetin from a calibration curve, which prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg ml-1 in methanol.

Chelating Activity: Chelating activity (Fe^{2+}) was measured by 2,2-bipyridyl competition assay according to the method described by Perumal and Klaus [22]. The reaction mixture containing 0.25 ml FeSO4 solution (1 M), 0.25 ml antioxidant solution, 1 ml Tris-HCl buffer (pH 7.4), 1 ml 2,2´-bipyridyl solution (0.1% in 0.2 M HCl) and 2.5 ml ethanol. The final volume made up 6.0 ml with distilled water. The absorbance was measured at 522 nm and used to evaluate chelating activity using disodium ethylene diamine tetracetate (Na2EDTA) as a standard.

DPPH Radical-Scavenging Activity: Free radical scavenging capacity of orange peel extracts was determined according to the previous reported procedure using the stable 2, 2-diphenyl-1- picrylhydrazyl radical (DPPH) as described by Ali *et al.* [23]. A freshly prepared DPPH solution in 0.5 ml ethanol were added to 3 ml of diluted each orange peel extract to start the radical antioxidant reaction. The final concentration was 100 µM for DPPH. The decrease in absorbance was measured at different intervals (i.e. 0, 0.5, 1, 3, 5, 10 and 15 min.) up to 50% at 517 nm. The remaining concentration of DPPH in the reaction mixture was calculated from a standard calibration curve. The absorbance measured at 5min of the antioxidant-DPPH radical reaction was used to compare the DPPH radical scavenging capacity of each extract.

Reducing Power Ability: The reducing power of orange peel extracts was quantified by the method described previously with minor modification. Orange peel extract (0, 1, 2, 3, 5, 7, 9 or 11 mg) in 1 ml methanol (80%) were mixed with 5 ml phosphate buffer (2 M, pH 6.6) and 5 ml potassium ferricyanide (1%). These mixtures incubated at 50°C for 20 min. 5 ml trichloroacetic acid (10%) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (5 ml) was mixed with 5 ml distilled water and 1 ml ferric chloride (0.1%). The absorbance of the pink color mixture was measured spectrophotometrically at 700 nm according to the method described by Perumal and Klaus [22]. Increased absorbance of the mixture indicates increased reducing power.

Statistical Analysis: The data were statistically analyzed by analysis of variance (ANOVA) and least significance difference (LSD) at a significance of probability 5 % [24].

RESULTS

Effect of Different Organic Solvents on Yield of Orange Peel Extracts (OPE): The tested organic solvents showed various yield percentages (8.27 - 28.32%) of orange peel extracts as shown in table 1. Also, from the same table, it is clear that the highest value was obtained in methanolic extract (28.32%) followed by ethanolic extract (27.96%).

Table 1: Effect of solvent type on % yield of orange peel extracts (OPE)

Organic solvents	Yield (mg / 10 ml)	% Yield of orange peel extract
Methanol	69.27	28.32
Ethanol	65.82	27.96
Dichloro methane	34.79	13.29
Acetone	49.20	18.21
Hexane	21.76	8.27
Ethyl acetate	58.27	24.92

Table 2: Effect of different solvents on TPC, TFC and chelating activity of produced OPE

Extract	TPC (mg/g)	TFC (µg/g)	Chelating Activity (µg/g) (as EDTA)
Methanol	165.38	28.36	1083
Ethanol	169.56	29.75	1097
Dichloro methane	98.64	17.39	734
Acetone	145.79	21.87	1021
Hexane	63.20	13.89	575
Ethyl acetate	85.27	18.36	937

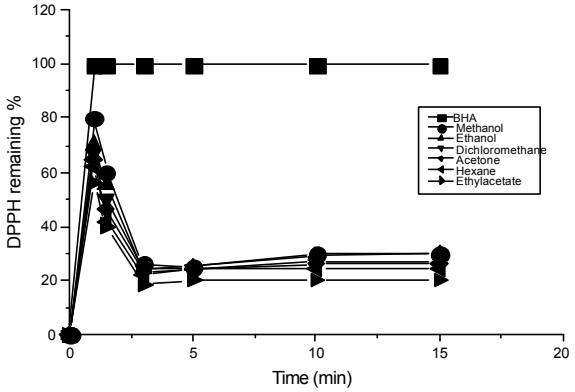

Fig. 1: Kinetic behavior of radical scavenging activity of orange peel extracts as assayed by the DPPH method

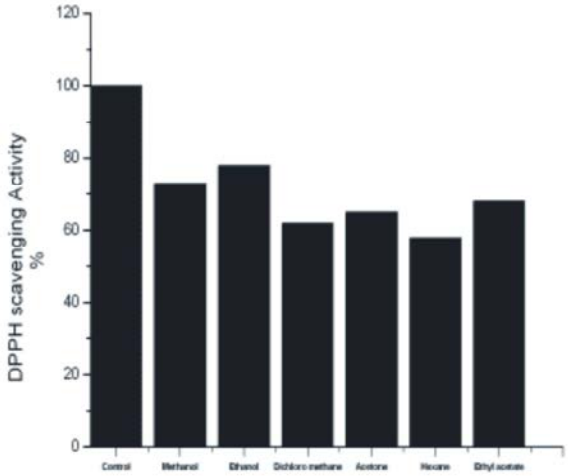

Fig. 2: DPPH radical scavenging activity of orange peel extracts at 5 min.

Effect of Different Solvents on TPC and TFC of Produced OPE: Table 2 shows that total polyphenols content (TPC) of the different orange peel extracts (such as gallic acid) in the range from 63.20 - 169.56 mg/g orange peel extract. While, total flavonoids content (TFC) was 13.89 - 29.75 µg/g (such as quercetin). Also, the same table indicats that TPC and TFC are relatively higher in ethanolic extract (169.56 mg/g and 29.75 µg/g, respectively), followed by methanolic extract (165.38 mg/g and 28.36 µg/g, respectively).

The chelating activity was measured against Fe2+ and reported as EDTA equivalents as shown in table 2. The EDTA equivalent ranged between 575 - 1097 µg/g of tested orange peel extracts. The highest value of chelating activity was found in ethanolic extract (1097 µg/g), while lowest value in hexane extract (575 µg/g).

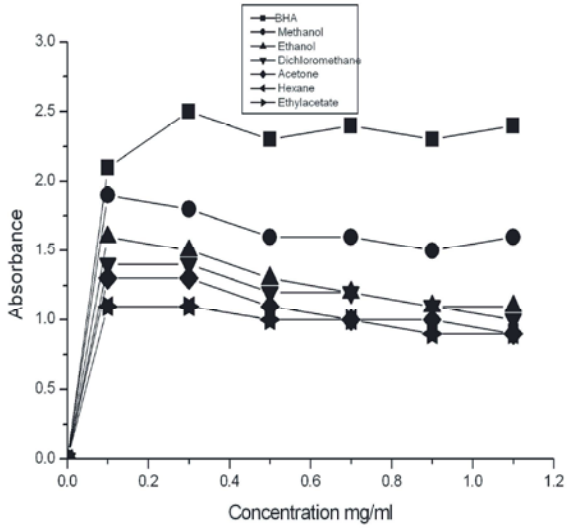

Fig. 3: Reducing power for various extracts of orange peel

Effect of Different Solvents on DPPH Radical-Scavenging Activity of Produced OPE: Fig. 1 illustrates a significant ($p < 0.05$) decrease in the concentration of DPPH due to scavenging activity of orange peel extracts. Maximum difference among the extracts was observed at 5 min of the reaction. The remaining % of DPPH radical at 5 min after initiation of reaction was 73.42, 78.14, 62.06, 65.38, 58.78 and 68.99% for extracts of methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate, respectively as shown in Fig. 2.

Effect of Different Solvents on Reducing Power Ability of Produced OPE: The reducing power of the extracts was compared with a known reducing agent BHA as shown in Fig. 3. The reducing power of the tested extracts was markedly lower ($p < 0.05$) than reducing power of BHA. However, among these extracts, the ethanolic extract of orange peel has shown the highest reducing power. Also, the reducing power of the extracts was slit increased with an increase concentration of the extract as shown in Fig. 3.

DISCUSSION

In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from the used plant raw material. Orange peel is one of the important dietary sources of antioxidant phenolics [4-7].

Calorimetrically analysis of polyphenolic and total flavonoid contents indicated that the ethanolic extract of orange peel had highly amounts of TPC and TFC and this in agreement with Ma *et al.* [25] who studied the physical and chemical characteristics of citrus peel and traced high amount of TP.

The metal chelating capacity is significant since, it reduces the concentration of catalyzing transition metal in lipid peroxidation. Moreover, the chelating agents, which form ó-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion, therefore it is an important parameter [26].

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 [27].

Regarding the reducing power, it is found that the amount of phenolic compounds was high in ethanolic extract of orange peel and there was a tight relationship between the amount of total phenolic content and the reducing power. These results were previously recorded [25,28] indicating that the reducing power of bioactive compounds is associated with antioxidant activity. Thus, it is necessary to determine the reducing power of phenolic constituents to elucidate the relationship between their antioxidant effects and the reducing power [29].

It could be concluded that, the solvent play a vital role in the extraction of the plant constituents. As methanol and ethanol are the highest polar among the solvents used. Therefore, they contain high yield of phenolic compounds and the highest antioxidant activity (% DPPH scavenging activity) if compared to other solvents extracts.

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