



Food and Dairy Research

<http://www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master>**ANTIOXIDANT ACTIVITY OF APPLE PEEL EXTRACTS PREPARED BY DIFFERENT SOLVENTS****Ahmed E. Hegazy***

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Received: 07/11/2017 ; Accepted: 03/12/2017

ABSTRACT: Crude extracts of apple peel were prepared by soxhelt extraction method with solvents methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate. Extracts were subjected for the measurement of total phenolic content (TPC) by Folin-Ciocalteu method as well as flavonoid content, chelating activity, reducing power and antioxidant/radical scavenging activity [1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity]. Results for all samples were in agreement with each other. The results revealed that all extracts of the apple peel exhibit antioxidant activity. These findings suggest that the apple peel extracts could act as potent source of antioxidants.

Key words: Apple peel, antioxidant activity, phenolic contents, flavonoids.

INTRODUCTION

Fruits and vegetables can be considered as an ideal source of natural antioxidants. It is credible to speculate that increasing consumption of these fruits intentionally will increase the intake of natural antioxidants, which may provide an alternative in the intervention of the aging process by protecting against oxidative damage. Various fruits may provide protection differently against oxidative stress since they are different in antioxidant capacity. We hypothesize that fruits with high antioxidant capacity are more effective than those with low antioxidant capacity in reducing oxidative damage associated with the aging process. Fruits are rich in antioxidants that help in lowering incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process. Antioxidants are substances which when present at low concentration are able to prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species. These reactive oxygen species mainly are reactive free radicals such as superoxide, hydroxyl, peroxy, alkoxy and non-radicals such as hydrogen peroxide, hypochlorous. They

scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi *et al.*, 2001).

One of the examples of fruit that contains high antioxidant contents is apple. It is important source of flavonoids and phenols which is consumed during whole year. Consumption of apples has been linked with the prevention of chronic diseases and with a lower incidence of cancer (Jakopic *et al.*, 2009). Apple consumption has also been associated with a decreased risk of coronary heart disease in women (Sesso *et al.*, 2003). Apples contain many types of phenolic derivatives and flavonoids. Moreover, the concentration of phenolic compounds may be affected by apple, cultivar and genus and also by extrinsic factors, such as soil, seasonality, agronomic factors, light exposure, *etc.* (Vieira *et al.*, 2011) Apple polyphenols have various *in vitro* bioactivities (*i.e.*, reduced risk of coronary heart disease. It is highly beneficial for our health due to its medicinal properties that act against diseases associated with oxidation and anti-aging (Balboa *et al.*, 2013).

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The purpose of this study was to evaluate apple peel as new potential source of natural antioxidants. In this study, the extracts of apple peel were prepared in methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate by soxhelt continuous extraction; The antioxidant activity of the extracts was assessed by modification of established assays, such as total phenolic content by Folin-Ciocalteu reagent; total flavonoids content, chelating activity by 2, 2-bipyridyl competition assay; antioxidant activity as free radical scavenging by DPPH and reducing power.

MATERIALS AND METHODS

Chemicals and Reagents

Folin-Ciocalteu reagent, methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate were purchased from E. Merck. Ferrous sulphate, Disodium ethylenediaminetetraacetate (Na₂EDTA), and butylated hydroxyanisole (BHA), were purchased from Fluka Riedel-de Haën. Quercetin, Gallic acid, 2,2-bipyridyl, HCl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich GmbH, Germany. All other chemicals and solvents were of the highest analytical grade and used as supplied.

Preparation of Extracts

Egyptian Anna apple (*Malus domestica* Borkh) fruits were harvested at Nubaria city, Egypt. Apples were washed, separated into peels and sliced into small pieces. The sample was oven dried at 50- 60°C to constant weight (Duh *et al.*, 1999).

Yield Estimation

Yield was estimated by reported method of Prashani *et al.* (2005). Each extract (10 ml) was measured into a pre-weighed aluminum dish. The samples were kept in an oven at 85°C for 24 hours then followed by placing in desiccator for 12 hours. The weight difference was used to calculate percentage yield as well as expressed in mg/10 ml.

Determination of total flavonoid content

Colorimetric aluminum chloride method was used for flavonoid determination (Ebrahimzadeh

et al., 2008). Briefly, 0.5 ml solution of each plant extracts in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg ml⁻¹ in methanol.

Determination of total phenolic content

Total phenolic compounds were determined by the Folin-Ciocalteu method (Ebrahimzadeh *et al.*, 2008). The extracted samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na₂CO₃ (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetry 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). Total phenol values are expressed in terms of gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound.

DPPH radical-scavenging activity

Free radical scavenging capacity of apple peel extracts was determined according to the previous reported procedure using the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) (Ali *et al.*, 2001). Briefly, a freshly prepared DPPH solution in ethanol (0.5 ml) was added to 3 ml of diluted each apple 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, 2, 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.

$$\text{DPPH remaining (\%)} = \frac{[\text{DPPH}] \text{ T}}{[\text{DPPH}] \text{ T}=0} \times 100$$

Where: T is the time interval.

Reducing power ability (RPA)

The reducing power of apple peel extracts was quantified by the method described previously (Perumal and Klaus, 2003) with minor modification. Apple peel extract (0, 1.0, 2.0, 3.0, 5.0, 7.0, 9.0, 11.0 mg) dissolved in 1 ml of 80% methanol were mixed with phosphate buffer (5.0 ml, 2.0 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%) ; the mixtures were incubated at 50°C for 20 min. A portion (5.0 ml) of trichloroacetic acid (10%) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (5.0 ml) was mixed with distilled water (5.0 ml) and ferric chloride (1.0 ml, 0.1%), and then absorbance of the pink colour mixture was measured spectrophotometrically at 700 nm. Increased absorbance of the mixture indicates increased reducing power. The experiment was conducted in triplicate and results were averaged.

Chelating activity

Chelating activity (Fe^{+2}) was measured by 2, 2'-bipyridyl competition assay (Pellegrini *et al.*, 1999). The reaction mixture containing 0.25 ml of FeSO_4 solution (1 mM), 0.25 ml of antioxidant solution, 1 ml of Tris-HCl buffer (pH 7.4), 1 ml 2,2'-bipyridyl solution (0.1% in 0.2 M HCl) and 2.5 ml of ethanol. The final volume was made up 6.0 ml with distilled water. The absorbance was measured at 522 nm and used to evaluate Fe^{+2} chelating activity using disodium ethylene diamine tetracetate (Na_2EDTA) as a standard.

Statistical Analysis

The results of this investigation are means of five measurements. To verify the statistical significance of all parameters the values of means \pm SD were calculated. To compare several groups, analysis of variance (ANOVA) was used. In the assessment of TRAP, the Spearman correlation coefficient (R) and P-value were used to show correlations and their significance. Linear regressions were also calculated. The P values of < 0.05 were adopted as statistically significant (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The yield of the extracts obtained by the soxhelt method was calculated as percent by weight of the apple peel. According to the chemical composition and polar nature of phenolic compounds, apple contains a relatively high percentage yield in ethanol and methanol while lower in hexane. Percentage yields in ethanol and methanol are comparable but have slight difference as shown in Table 1.

The phenolic compounds may contribute directly to the antioxidant action therefore, it is necessary to investigate total phenolic content. The total phenolic content was determined by following a modified Folin-Ciocalteu reagent method. In Table 2 the results were expressed as gallic acid equivalent. TPC was in the range of 63.90-170.56 mg/g of the apple peel extracts. The amounts of total phenolic compounds were higher ($p < 0.05$) in ethanol extract (170.56 mg/g) while they were lowest ($p < 0.05$) for hexane (63.90 mg/g). Using a standard curve of gallic acid ($R^2 = 0.999$). All results coincide with those of total antioxidant capacity. In other words, the spice extract sample shows a tendency to have high phenolic content.

Using the AlCl_3 reagent and quercetin as standard ($R^2 = 0.9996$), the total flavonoids are in the range from 14.89-31.75 $\mu\text{g/g}$ of quercetin equivalent (Table 2). The highest value ($p < 0.05$) for the ethanol was 31.75 $\mu\text{g/g}$ and the lowest ($p < 0.05$) was 14.89 $\mu\text{g/g}$ of the apple peel with the following decreasing order of the extract ethanol $>$ methanol $>$ acetone $>$ ethyl acetate $>$ dichloromethane $>$ hexane. Flavonoids are not easily detectable therefore, in the extract AlCl_3 was used as complexing reagent.

The chelating activity was measured against Fe^{+2} and reported as EDTA equivalents as shown in Table 2. The difference ($p < 0.05$) in chelating activity was observed among the extract. The highest chelating activity was observed in ethanol. The EDTA equivalent was in the range of 1097-575 $\mu\text{g/g}$ of apple peel extracts (Table 2).

According to Ilhami *et al.* (2005) metal chelating capacity is significant since it reduces the concentration of catalyzing transition metal

Table 1. Percentage yield of apple peel extract in different organic solvents as well as in mg/10 ml.

Organic solvent	Yield mg / 10 ml	Yield of apple peel extract (%)
Methanol	70.27	29.32
Ethanol	66.82	28.96
Dichloro methane	35.79	14.29
Acetone	49.90	18.91
Hexane	22.76	9.27
Ethyl acetate	59.27	25.92

Table 2. Total phenolic content (TPC), flavonoid content (FC) and chelating activity of organic solvent extracts of apple peel expressed as gallic acid, quercetin and Na₂EDTA equivalent, respectively

Sample	TPC gallic acid eq. (mg/g of apple peel)	FC Quercetin eq. (µg/g of apple peel)	Chelating activity EDTA eq (µg/g of apple peel)
Methanol	165.58	28.56	1093
Ethanol	170.56	31.75	1099
Dichloro methane	98.94	17.89	784
Acetone	149.79	22.87	1031
Hexane	63.90	14.89	579
Ethyl acetate	86.27	18.96	794

Data are mean (n = 3) ± SD (n = 3), (p < 0.05).

in lipid peroxidation. Moreover, the chelating agents, which form δ -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.

The antioxidant capacity of the apple peel extracts were analyzed by using the free radical scavenging (DPPH^o) and the ferric reducing antioxidant power (FRAP) methods. The DPPH^o test is the oldest indirect method for determining the antioxidant activity, which is based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols. 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. As Fig.1 illustrates a significant decrease (p<0.05) in the concentration of DPPH due to scavenging activity of apple peel extract. Maximum difference among the extract was observed at 5 min of the reaction and the remaining amount (%) of DPPH radical at 5 min after initiation of reaction as shown in Figure 2 was 78.14, 73.42, 62.06, 65.38, 58.78 and 68.99 for ethanol, methanol, dichloromethane, acetone, hexane and ethyl acetate, respectively. The high amount of the phenolic compounds and reducing power having the highest percent DPPH scavenging activity was shown by the ethanol extract, and the second highest activity was determined in the methanol while lowest in hexane.

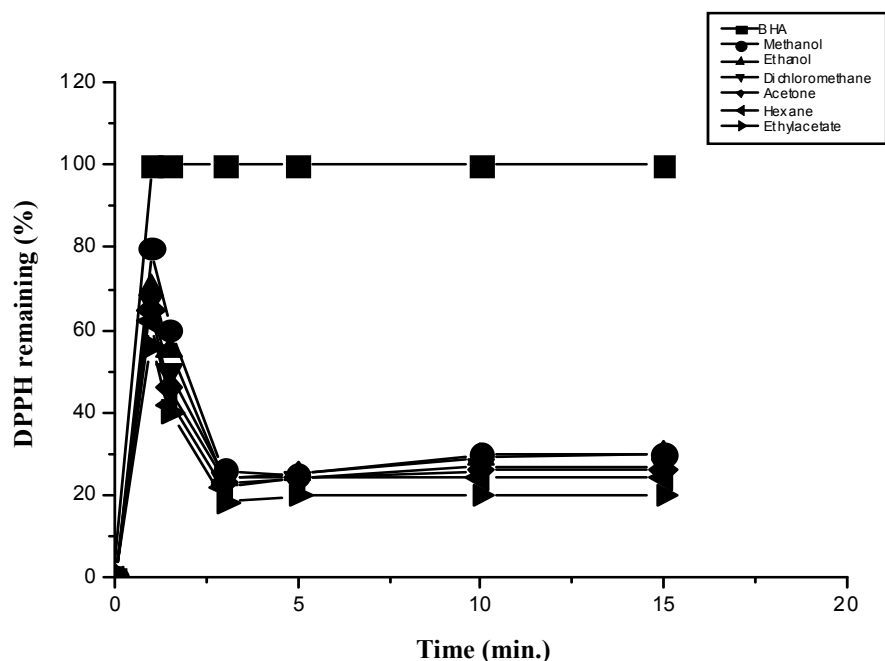


Fig. 1. Kinetic behavior of radical scavenging activity of cumin extracts as assayed by the DPPH method. The final DPPH concentration was kept 100 μ M in all reaction mixtures. Values are mean (n =3), (P < 0.05)

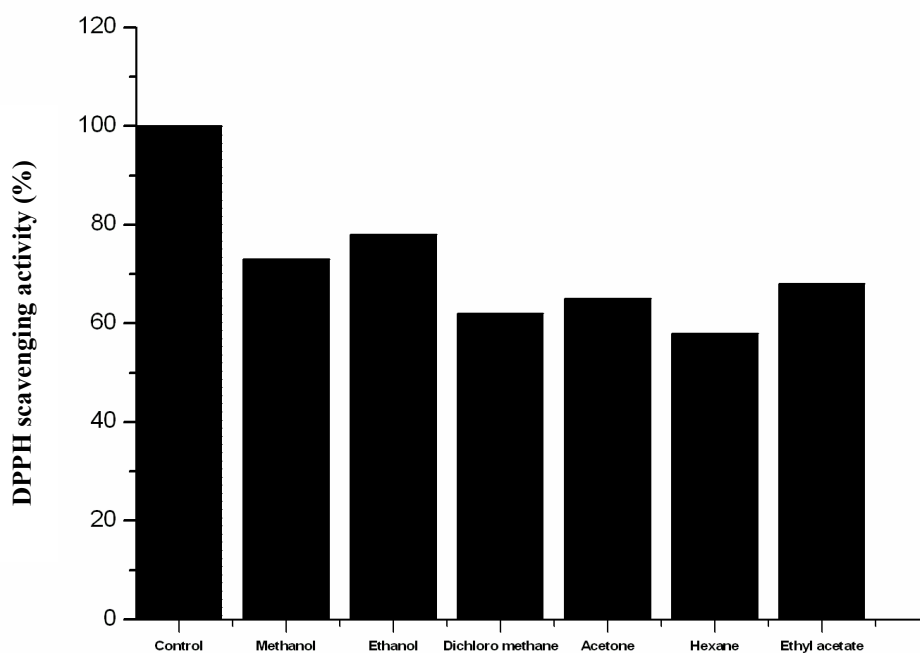


Fig. 2. DPPH radical scavenging activity of apple peel extract at 5 min. Vertical bars represents the standard deviation of each data point. Values are mean (n = 3), (P < 0.05)

It has been reported 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 their reducing power (Shan *et al.*, 2005). The reducing power of the extracts increases ($P < 0.05$) with an increase in the amount of the extract as shown in Fig. 3. The amount of the phenolic compounds was high in ethanol extract of apple peel, therefore; similar results were obtained in reducing power activities. Hence, by correlating these results; we can suggest that there may be relationship between the amount of total phenolic content and reducing power.

The reducing power of the extracts was compared with a known reducing agent BHA. The reducing power of the extract was markedly lower ($P < 0.05$) than that of BHA. However,

among these extracts the ethanol extract of apple peel has shown the highest ($P < 0.05$) reducing power.

Conclusion

From this study, it could be concluded that the solvent play a vital role in the extraction of the plant constituents. As methanol and ethanol are highly polar among the solvents used, therefore, they contain high yield of phenolic compounds as compared to the other solvents. An ethanolic extract of apple peel was shown highest antioxidant activity (% DPPH scavenging activity). The antioxidant activity could be correlated with the polyphenolic components present in the extract. The results obtained from these methods provide some important factors responsible for the antioxidant potential of apple peel.

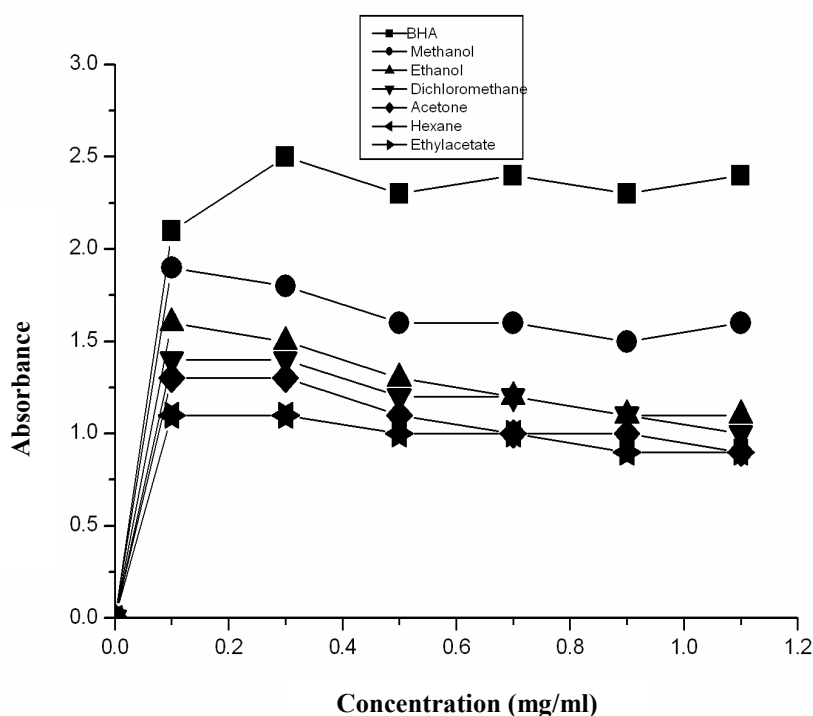


Fig. 3. Reducing power of ethanol, methanol, dichloromethane, acetone, hexane and ethyl acetate extract of apple peel. All data is reported as mean \pm S.D (n = 3) statistically significant as $P < 0.01$

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النشاط المضاد للأكسدة لمستخلصات قشور التفاح المحضرة بمذيبات مختلفة

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أجريت هذه الدراسة بغرض تقدير محتوى مستخلصات مختلفة لقشر التفاح (مثل مستخلص كل من الميثانول والإيثانول والداي كلوروميثان والأسيتون والهكسان والإيثيل أسيتات) من الفينولات العديدة والفلافونيدات وإمكانية الاستفادة من هذه المستخلصات كمضادات أكسدة طبيعية في الأغذية، كما تم أيضا دراسة تأثير نوع المذيب المستخدم في الاستخلاص على العوامل المسببة للنشاط المضاد للأكسدة، وقد أشارت نتائج التحليل اللوني أن المستخلصات المختلفة لقشر التفاح كانت عالية المحتوى من الفينولات العديدة والفلافونيدات، خاصة مستخلصات الميثانول والإيثانول (١٦٥,٥٨، ٢٨,٥٦، ١٧٠,٥٦ و ٣١,٧٥ على التوالي)، كما أوضحت نتائج تقييم مستخلصات قشر التفاح كمضادات أكسدة طبيعية باستخدام طريقة DPPH على وجود فروق معنوية بين تلك المستخلصات مشيرة إلى أن كفاءة المستخلص الإيثانولي كانت الأعلى مقارنة بالمستخلصات الأخرى

المحكمون :

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