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RESEARCH ARTICLE

Evaluation of Antioxidant Activity of Apple Peel and Pulp Extracts by Using Different Solvents

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Abstract: Evaluation of antioxidant activity of apple peel and pulp extracts by using different solvents was studied. Apple variety used for extraction was Gala. Apple peel and pulp extracts were prepared by using methanol, chloroform: acetone (4:1) and ethyl acetate solvents**.** The antioxidant activities of these extracts were determined by DPPH (1, 1- Diphenyl - 2- picryl hydroxyl) assay and RPA (reducing power assay) method. On comparing the activities of peel and pulp it was found that the activity of peel extracts was higher. It was found that free radical scavenging activity of both apple peel and pulp crude extracts was significantly higher in methanol extract among all the extracts studied. Reducing power assay (RPA) also gave significant results with methanol extract. All the extracts showed lower antioxidant activity compared to Gallic acid when used as standard.

Keywords: DPPH - (1, 1- Diphenyl - 2- picrylhydrazyl) assay, Reducing power assay, Antioxidant activity, Apple peel and pulp

Introduction

Fruits and vegetables contain high antioxidant which is beneficial for our health. In recent years, a wide variety of fruit products with beneficial health effects have been developed and marketed. However, only limited information on the nutritional value and bioactive compounds of tropical fruits, especially the more exotic species is currently available[1].

One of the examples of fruit that contains high antioxidant contents is apple *(malus domestica)*. It is important source of flavonoids and phenols which is consumed by us whole year. Consumption of apples has been linked with the prevention of chronic diseases and with a lower incidence of cancer[2]. Apple consumption has also been associated with a decreased risk of coronary heart disease in women[3]. Apples contain many types of phenolic derivates and flavonoids. Moreover, the concentration of phenolic compounds may be affected by apple variety, cultivar and genus and also by extrinsic factors, such as soil, seasonality, agronomic factors, light exposure, *etc*[4]. Apple polyphenols have various *in vitro*

bioactivities, possibly in combination with dietary fibre (*i.e*. reduced risk of coronary heart disease)[5]. It is highly beneficial for our health due to its medicinal properties that act against diseases associated with oxidation and anti-aging[6].

Recent studies has confirmed that the unripe apples along with its seeds and peels are a great source of polyphenols[7]. Free radicals are normally generated in substantial amounts as a by-product of various internal metabolic processes in aerobic organisms such as phagocytosis, neutrophiles defence, auto oxidation of catecholamine and carboxylation or hydroxylation reactions. These free radicals are neutralized by antioxidants, as a result it prevents the oxidative stress that may lead to oxidation related diseases[8].

Anthocyanins pigments belong to the group of flavonoids has attracted great interest due to the wide biological activities, including its antioxidant, anti-inflammatory and anticarcinogenic properties[9]. There is higher amount of phenolic compounds and ascorbic acids in the peel than in the pulp for most of the fruits[10]. The skin usually contains higher bioactive compounds in order to protect the inner materials from insects and microorganisms[11]. Therefore the objective of this study was to compare antioxidant activity of apple peel and pulp extracts by DPPH and Reducing power assay method.

Experimental

Ethanol, methanol, chloroform, acetone and ethyl acetate, DPPH, potassium ferricynide, trichloro acetic acid and ferric chloride used were of Merck and Loba fine.

Sample preparation

The apple fruits were collected from the local market of Allahabad. Apples were washed, separated into pulps and peels and sliced into small pieces. The sample was oven dried at 50- 60 ºC to constant weight[12].

Prepation of extracts

Apple peel and pulp extracts were prepared by the Soxhlet method[13]. 25 g of dried peel and 45 g of dried pulp sample were taken in soxhlet for different solvents [methanol, chloroform: acetone (4:1)] and ethyl acetate] and the extraction was carried out twice for 8 h at 60 ºC separately. The solvents in the extracts were removed using distillation unit at 40 ºC. The standard extracts were obtained and sealed with aluminum foils and stored in the refrigerator at 4 ºC until required for Antioxidant activity.

DPPH free radical scavenging assay

The hydrogen atom or electron donation capacity of the extracts was measured as a decrease in absorbance of DPPH. The scavenging ability of apple peel and pulp extracts were determined based on the method given by Yamaguchi[14]. The reaction between antioxidant compounds with the stable DPPH radical will caused reduction in absorbance and decolourisation of DPPH to light yellow[14]. To 1.5 mL of methanolic DPPH solution (0.1 mM) was introduced varying concentrations of the extract in methanol [0.1 mg/mL (100 µgm/mL) – 5.0 mg/mL (5000 µgm/mL)]. A control sample was prepared without extract containing DPPH solution in a appropriate volume. Methanol was used as a blank. The mixtures obtained were shaken well and left for 20 minutes at room temperature and the absorbance of the resulting solutions were taken at 517 nm against a blank in UV visible spectrophotometer. The radical scavenging activity was measured as a decrease in the absorbance of DPPH.

$$\% \text{ scavenging activity} = 1 - \frac{\text{Abs sample} \times 100}{\text{Abs Control}}$$

Reducing power assay

In this assay, Fe^{3+} / ferricyanide complex is reduced to the ferrous form by antioxidants. The Fe^{2+} formed is monitored by measuring the formation of Prussian blue colour at 700 nm[15]. Different concentrations of extract (200, 400, 600 and 800 µg/mL) were mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] in test tubes. The mixture was incubated for 20 min at 50 ^oC. At the end of the incubation 2.5 mL of tri-chloroacetic acid was added to the mixtures followed by centrifuging at 500 rpm for 10 min. The upper layers (2.5 mL) were then mixed in 2.5 mL distilled water and 0.5 mL of ferric chloride and the absorbance was measured at 700 nm. The reducing power tests were done in triplicates. Increase in absorbance of the reaction mixture indicated the reducing power of sample.

Results and Discussion

The DPPH free radical scavenging activity and reducing power activity of apple peel and pulp extracts were determined for different solvents (methanol, chloroform: acetone (4:1) and ethyl acetate). Gallic acid was taken as standard.

The results in Table 1 and Figure 1 reveal that percent inhibition increases with increasing concentration of extract whereasTable 2 and Figure 2 reveal that reducing power activity of extract increases with increase in concentration.

Table 1. DPPH Free radical scavenging activity of peel and pulp extracts of apple

Conc. µg/mL	% inhibition (methanol peel) Mean ± S.D	% inhibition (methanol pulp) Mean ± S.D	% inhibition (chloroform: acetone peel) Mean ± S.D	% inhibition (chloroform: acetone pulp) Mean ± S.D	% inhibition (ethyl acetate peel) Mean ± S.D	% inhibition (ethyl acetate pulp) Mean ± S.D	% inhibition Gallic Acid Mean ± S.D
200	41.57	26.39	36.80	18.75	20.07	15.65	71.19
400	47.19	32.16	38.93	31.77	33.33	28.18	72.01
600	50.00	40.41	45.33	38.80	43.46	34.00	72.42
800	58.98	48.86	57.86	45.83	48.25	43.40	73.66

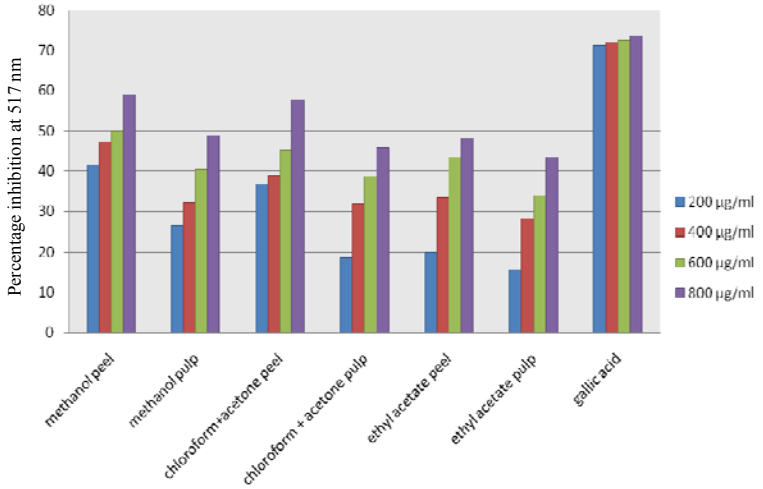

Figure 1. DPPH Free radical scavenging activity of peel and pulp extracts of apple

Table 2. Reducing Power Activity of peel and pulp extracts of apple

Conc. µg/mL	(methanol Peel extract) Mean ± S.D	(methanol Pulp extract) Mean ± S.D	(chloroform: cetone(1:4) peel extract) Mean ± S.D	chloroform: acetone(1:4) pulp extract Mean ± S.D	Ethyl acetate Peel extract Mean ± S.D	Ethyl acetate Pulp extract Mean ± S.D	Gallic Acid Mean ± S.D
200	2.278	2.077	1.913	1.795	1.799	1.706	2.404
400	2.352	2.173	1.995	1.831	1.886	1.786	2.457
600	2.414	2.282	2.080	1.870	2.034	1.841	2.499
800	2.492	2.323	2.176	1.915	2.155	1.904	2.601

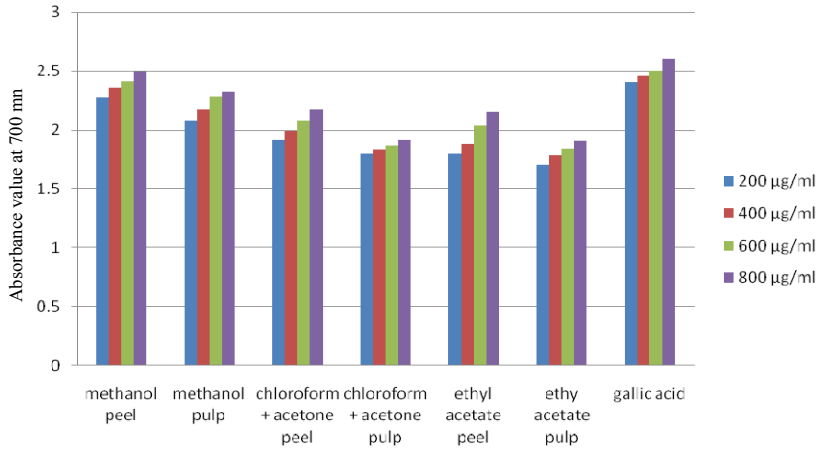

Figure 2. Reducing Power Activity of peel and pulp extracts of apple

Among extracts peel extracts showed higher antioxidant and reducing activity than pulp extracts. This may be due to the presence of different antioxidant compound *viz*. apple pulp *viz*. catechin, procyanidin, caffeic acid and chlorogenic acid whereas the peel contains aforementioned substances as well as flavonoids, not present in pulp, such as quercetin glycosides and cyanidin glycosides which makes peels more effective than pulp[16].

While among the different solvent extracts the high antioxidant and reducing activity was measured in methanol extract of apple. The extract obtained from polar solvents had higher concentrations of phenol while the extracts obtained from low polar solvents were found to have small concentrations[17].

Summary and Conclusion

The dried peel and pulp sample of *Malus domestica* were extracted using three solvents methanol, chloroform: acetone (4:1) and ethyl acetate. Antioxidant activity was taken by using two methods DPPH free radical scavenging activity and reducing power assay method. It was also observed that the two methods (DPPH free radical scavenging activity and reducing power assay) showed similar trends in antioxidant activity for a particular extract.

Further, the potential of apples must be explored more and more, in order to develop an alternate therapy for the treatment of infections caused by free radicals and microorganisms Bacteria and the present study also suggest that this apple may be exploited for health supplement.

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