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DNA PROTECTIVE ACTIVITY OF PEELS OF SOME VEGETABLES WASTES

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ABSTRACT

The present study was conducted by using kitchen wastes like peels of different vegetables e.g. cucumber, potato, carrot, chickpea, cauliflower and pea, to evaluate the phytochemical constituents, DNA protection ability and free radicals scavenging activity. Phytochemicals tests showed the presence of carbohydrates, amino acids, glycosides, alkaloids, tannins and steroids in all samples while phlobatannins, anthraquonines and saponins were absent. The DNA protection of samples was checked by DNA protection assay using plasmid pBR322. Cauliflower and cucumber showed best protection at each dilution. DPPH free radicals scavenging assay was used to evaluate the antioxidant activity of peel extract of vegetables. Carrot showed the highest antioxidant activity of about (84%) at 10 mg/ml, followed by pea and chickpea with 75% and 79% respectively. It is therefore concluded that peels of cauliflower and cucumber in terms of DNA protection activity, and carrot, pea and chick pea in terms of DPPH free radicals scavenging assay can be helpful and all these peels which are wasted can be utilized for medicinal and other purposes.

Keywords: Phytochemicals, DPPH assay, Antioxidant, ROS, kitchen waste.

INTRODUCTION

Organisms obtain energy necessary for biological processes by a process called oxidation, which is the cellular process of metabolism. This lead to free radical formation, commonly known as reactive oxygen species (ROS). Nature has provided each cell with protective mechanisms against all kind of effects caused by free radicals which can be harmful. This is generally called the antioxidant defense mechanism. This helps in preventing many degenerative and chronic diseases such as heart diseases, diabetes mellitus, cancer, Alzheimer and aging (Li *et al*., 2007). Intake of antioxidants by using fruits and vegetables in diet should protect cells against the reactive oxygen species and also help in decreasing the DNA damage caused by oxidation (Moller *et al.*, 2003; Nisar *et al*., 2015). Various plant phytochemicals such as tannins, phenolic compounds and flavonoids are reported to have important antioxidant activity against a wide variation of free radicals (Koleckar *et al*., 2008; Kirmizibekmez *et al*., 2009; Choudhary & Swarnkar, 2011). These active compounds can be isolated and developed as natural drugs for the prevention and treatment of free radical related disorders.

Vegetable wastes of cucumber, cauliflower, chickpea, carrot, potato and pea are examined in this study. The water contents of cucumber (*C. sativus)* are very high as compared to calories. It has potential antidiabetic, lipid lowering and antioxidant activity. According to Mukerjee *et al.* (2013), different parts of *C. sativus* have a therapeutic application, pharmacological and phytochemical profile. Potatoes are the predominant staple for many countries, but in

this study they account for only about 2% of the food energy supply. According to Burlingame *et al., (*2009), in potato there are several phytochemicals such as flavonoids, polyamines, carotenoids and phenols, which are highly desirable in diet because of their beneficial effects on human health. Cauliflower is considered a food of high nutritional value and indicates that its quality is similar to the stability of fatty acids. Samples of the 'Verde di Macerata' variety were analyzed for the quality parameters, fatty acid composition and stability for antioxidant properties. The results showed a decrease in ascorbic acid content, and an increase of dry and fatty matter, polyphenols and antioxidant activity (Scalzo *et al*., 2007). Carrots were used for investigation of the interaction of cultivars, as well as the effects and mineral supply on the nutritional quality (vitamin C, antioxidant potential and phenolic acids). In 'Nutri-Red' carrots, high calcium levels decrease the lycopene content. The major determinants of nutritional quality of the carrots were both cultivars and mineral supply. Nutritional quality can be enhanced by manipulating mineral nutrient application levels of micronutrients, since this leads to an increase in the antioxidant properties (Davinder *et al*., 2012). Pea varieties with optimal functional and health effects may be a convenient route of measurement of TAC (Nilsson *et al*., 2004).

Therefore, the main objectives of the study are to determine phytochemical constituents and DNA protective activity and also to determine the scavenging activity of peel extract of several vegetables (cucumber, cauliflower, chickpea, carrot, potato and pea).

MATERIALS AND METHODS

The fresh peels of vegetables (cauliflower, carrot, chickpea, cucumber, pea, and potato) were collected from kitchen wastes and were air dried under shade. The peels were then cut in to small pieces and ground by an electric grinder into fine powder. Crude methanolic extracts (CME) were prepared by dissolving carefully weighed 5 g of powdered samples in 50 ml of methanol. It was then kept at room temperature by shaking it after every 2 h for one day. Thereafter, it was filtered by using Whattmann filter paper No. 1. The crude methanolic extracts were used for the phytochemical analysis by using procedures described by Sofowora, (1993). DNA protection assay was conducted by following the protocol described by Tian and Hua (2004). The antioxidant activity of all the six samples was done by following the protocol described by Kulisic *et al.* (2004).

RESULT AND DISCUSSION

In this study, peels of different vegetables were used to find out their antioxidant and DNA protection ability. For this purpose, phytochemical screening, plasmid DNA protection assay and (DPPH) 2, 2-diphenyl-1-picryl-hydrazyl free radical scavenging assay were used to evaluate their antioxidant activity. The results of phytochemical analysis of extracts of peels are shown in Table 1. The amino acid, carbohydrate, and tannins were found in all extracts of peels of vegetables, while alkaloids were detected in extracts of potato, cucumber, cauliflower, pea and absent in carrot and chickpea. Glycosides and steroids were detected only in extracts of potato and cauliflower while absent in cucumber, carrot, chickpea and pea. Phlobatannins were found to be present only in extract of potato. The anthraquinones and saponins

Table 1: Phytochemical constituents of different vegetables wastes used in the study.

	Potato	Cauliflower	Cucumber	Pea	Carrot	Chickpea
Tannins	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+
Alkaloids	+	+	+	+	-	-
Glycosides	+	+	-	-	-	-
Steroids	+	+	-	-	-	-
Phlobatannins	+	-	-	-	-	-
Anthraquanine	-	-	-	-	-	-
Saponins	-	-	-	-	-	-

(+) sign indicate the presence of compound and (-) sign shows the absence of compound.

were bsent in all the samples of peel extracts. Hu, C. (2012), conducted phytochemical analysis on different vegetables including carrot, which determined the presence of phenolic acids, isoflavones, flavonols and glucosinolates. The results of our study showed the presence of tannins, amino acids and carbohydrate. Hence, the recent research showed that the complex mixture of phytochemicals in vegetables provides a better protective effect on health than a single phytochemical (Chu *et al*. 2004).

The DNA protective ability of the methanolic extract of different peels of vegetables was conducted by using DNA protection assay based on photolysing H_2O_2 and $FeSO_4$. In this assay, plasmid pBR322 was used along with four dilutions (1000 ppm, 100 ppm, 10 ppm and 1 ppm) of different samples. After the one hour incubation period, the samples were loaded along with positive and negative control by using each fraction of sample respectively (1000 ppm, 100 ppm, 10 ppm, 1ppm) on agarose gel electrophoresis. Normal pBR322 of control series showed 2 bands in agarose gel electrophoresis. The faster moving band represented the native form of supercoiled circular DNA (scDNA) and the slower moving band corresponded to the open circular form (ocDNA). The results are shown in Figure 1 and 2.

DPPH assay was performed for the evaluation of the antioxidant activity of peel extracts of different vegetables. The carrot showed the highest antioxidant activity of 84% at 10 mg/ml. Pea and chick pea also showed a high antioxidant activity at 75% and 79%, respectively, while potato, cucumber and cauliflower showed little antioxidant activity having 3%, -20% and 10% respectively. The results are shown in Table 2. The antioxidant activity for leaves extracts of potato was reported by Friedman (1997) as a percentage of free radical DPPH scavenging activity. Muller *et al*. (2011) worked on oil samples and several juices of carrots, tomatoes and oranges to assess the antioxidant capacity of carotenoids and xanthophylls. Carotenoids of carrot did not show any DPPH free radical scavenging activity, while in the current study carrot showed high DPPH free radical scavenging activity. The difference in the result may be due to the reason that

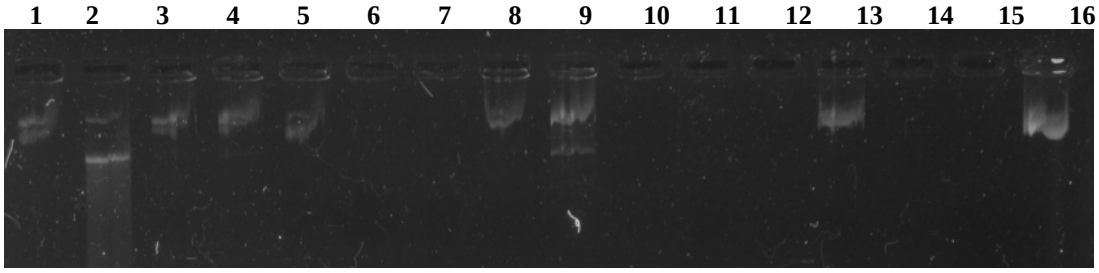

Figure 1: Plasmid DNA Protection Assay. Protection assay mixture contained 3 µl of 2mM$FeSo_4$, 4 µl of H_2O_2, 5 µl of PBS, 3µl of Plasmid with different concentrations of methanolic extract of various vegetables. Methanolic extract of cauliflower having 1000 ppm,100 ppm, 10 ppm and 1 ppm **Lanes 1-4**, Methanolic extract of carrot having 1000 ppm, 100 ppm, 10ppm and 1 ppm in **Lanes 5-8**, Methanolic extract of pea having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in **Lanes 9-12**, Methanolic extract of chickpea having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in **Lanes 13-14,** positive control (3 µl of 2mM$FeSo_4$, 4 µl of H_2O_2, 5 µl of PBS, 3µl of Plasmid) in **Lane 15,** negative control (3 µl of Plasmid, 5 µl of PBS) in **Lane 16.**

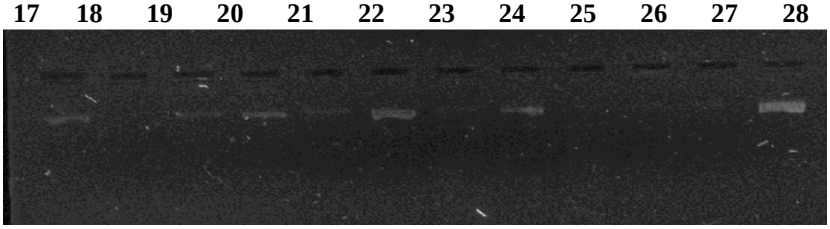

Figure 2: plasmid DNA protection assay. Protection assay mixture contained 3 µl of 2mM$FeSo_4$, 4 µl of H_2O_2, 5 µl of PBS, 3µl of Plasmid with different concentrations of methanolic extract of various vegetables. Methanolic extract of chickpea having 10 ppm and 1 ppm in **Lanes 17-18**. Methanolic extract of cucumber having 1000 ppm, 100ppm, 10 ppm and 1 ppm in **Lanes 19-22**. Methanolic extract of potato having 1000 ppm, 100 ppm, 10 ppm and 1 ppm in Lane **23-26**. Positive control (3 µl of 2mM$FeSo_4$, 4 µl of H_2O_2, 5 µl of PBS, 3µl of Plasmid) in **Lane 27** and negative control (3 µl of Plasmid, 5 µl of PBS) **Lane 28.**

Muller *et al.* (2011) estimated the antioxidant activity of xanthophylls and carotenoids isolated from whole juices while in the present study the whole peel extract was used. Cabello-Hurtado *et al*. (2011) studied the antioxidant activity of cauliflower leaves by using different *in vitro* assay. The results of this study were similar with the current study using the same part of the vegetable and showing a little DPPH free radical scavenging activity.

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Table 2: Result of DPPH assay performed.

Samples	Dilutions			
	10 mg/ml	**5 mg/ml**	**2.5 mg/ml**	**1.25 g/ml**
Pea	75%	-	-	-
Chickpea	79%	-	-	-
Cauliflower	10%	-	-	-
Potato	3%	-	-	-
Cucumber	-20%	-	-	-
Carrot	84%	58%	-43%	-20%

More than 80% activity of DPPH is shown by carrot extract as compare to other samples. Therefore, further dilutions of carrot extract were used for further evaluation. (-) sign shows no dilutions were prepared.

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