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Research Article

In Vitro Evaluation of Antioxidant and Antimicrobial Activity of Carrot Peel

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ABSTRACT
The objectives of the study were to assess the total phenolic content and flavonoid content, and to investigate the antioxidant and antimicrobial potential of carrot peel extracted with methanol and acetone. Total Phenolic content and Flavonoid content was higher in carrot peel acetone extract (AE) i.e. 106.36 µg/mg of GAE and 27.83 µg/mg of QE respectively than methanol extract (ME). Carrot peel acetone extract showed greater antioxidant capacity for DPPH radical scavenging activity whereas methanol extract showed better result for FRAP reducing power and Phosphomolybdenum assay. Both AE and ME could inhibit the growth of *Shigella flexneri, E coli, Staphylococcus aureus* and *Klebsiella pneumonia*. This study substantiates the potential of carrot peel to be used as a novel functional food ingredient, natural antioxidant and antimicrobial agent.

Keywords*: antimicrobial potential, carrot peel, Flavonoid, Phenolic content.*

INTRODUCTION

Vegetables and some fruits yield between 25% and 30% of nonedible products, which include skins and seeds that normally have no further usage and are commonly wasted or discarded[1,2]. The byproducts of plant food processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds which may be used because of their favourable technological or nutritional properties[3]. The by-products may still contain many valuable substances, such as, pigments, sugars, organic acids, flavours and bioactive compounds with antioxidant and antimicrobial activities, as well as being valuable sources of dietetic fibre[4]. Growing body of research evidence proves that the content of phytochemical compounds is higher in peel and seeds with respect to the edible tissue of fruits and vegetables. For eg. the total phenolic compounds in the peels of lemons, oranges, and grapefruits were 15% higher than that of the pulp of these fruits[5].

Carrot (*Daucus carota*) is classified as vitaminized food as it is rich in β-carotene, ascorbic acid and tocopherol[6]. Carrot is also a significant source of phenolic compounds such as hydroxycinnamic acids and derivatives[7], para-hydroxybenzoic acids[8] and polyacetylenes[9,10]. Due to appreciable level of variety of different compounds present, carrots are considered as a functional food with significant health promoting properties[11]. Carrot processing residues e.g. peels, pomace, are usually discarded or used as animal feed which are high in phenolics and antioxidant properties, could be considered for value-added utilization. The studies on evaluating the phenol content of carrot revealed the peel had higher content of phenolics than the flesh. Kähkönen et al.,[12] found that carrot peel and flesh contained 6.6 and 0.6 mg gallic acid equivalent/g dry weight respectively. Phenolic content in different tissues decreased from peel, phloem to xylem, similarly antioxidant and radical scavenging activities in different tissues decreased in same order as the phenolic content and correlated well with total phenolic contents[7].

Although studies have been reported on the antioxidant and antimicrobial properties of the carrot peel, it should be noted that result may vary according to the geographical location, soil in which the plant is grown, season, varieties and method of extraction. Therefore the current study was undertaken to elucidate antioxidant and antimicrobial properties of the carrot peel so as to validate its use as nutraceutical and as food preservative.

MATERIALS AND METHODS

Preparation of carrot peel extracts

Healthy, disease free and mature carrots were purchased from the local market. Cleaned and washed carrots were manually peeled using a sterilized peeler and the peels were then shade dried at room temperature for 5 days. The shade dried carrot peels were powdered in a laboratory blender and was kept in airtight bottles until further use. The powdered carrot peel was soaked in methanol and acetone for 72 h by maceration technique. The supernatants were filtered through Whatman No.1 filter paper and concentrated using rotary evaporator. The dry residue was preserved at 5°C in airtight bottles until further use.

Estimation of total phenolic content

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Figure 1: Carrot (*Daucus carota*) and powdered carrot peel.

Table 1: Total Phenolic and flavonoid content of carrot peel extract.

Extracts	Total Phenolic content	Total Flavonoid content
Methanol	19.94 µg/g of GAE	5.39 µg/g of QE
Acetone	106.36 µg/g of GAE	27.83 µg/g of QE

Table 2 a: DPPH activity of carrot peel acetone extract.

S.No	Concentration (µg/µL)	Acetone extract % of Inhibition
1	50	32.82
2	100	51.90
3	150	72.51
4	200	83.97
5	250	95.38
6	300	96.95

Table 2 b: DPPH activity of carrot peel acetone.

S.No	Concentration (µg/µL)	Methanol extract % of Inhibition
1	100	7.45
2	200	10.78
3	300	20.28
4	400	21.69
5	500	27.98
6	600	34.91

Table 3: FRAP reducing power assay of carrot peel acetone and methanol extract.

S.No	Concentration (µg/µL)	Absorbance at 700 nm	
		Acetone extract	Methanol extract
1	20	0.09	0.14
2	40	0.46	0.52
3	60	0.66	0.67
4	80	0.70	0.80
5	100	0.85	4.52
6	120	0.86	5.45

Total phenolic content of the carrot peel extracts (Methanol and Acetone) were estimated by the Folin-Ciocalteau reagent method[13]. Hundred µL of extracts (1 mg/mL) were made up to 1 mL by methanol and mixed with 1 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water) and after 5 min one mL of 20% sodium carbonate (Na_2CO_3) was added. The mixture was incubated at room temperature for 30 minutes and the absorbance was measured at 760 nm by spectrophotometer. The total phenolic content was expressed in terms of Gallic acid equivalent (µg/mg of extract), which is a common reference standard.

Estimation of total Flavonoid content

The total flavonoid content of the carrot peel AE and ME was determined by the Alumininum Chloride reagent method[14]. The extract (500 µg/mL) was made up to one mL by methanol and were mixed with 0.5 mL of 5% sodium nitrate ($NaNO_2$) solution and allowed to stand for 5 min. Then 0.3 mL of 10% Alumininum Chloride ($AlCl_3$) solution was added and the mixture was allowed to stand for further 5 min. Finally, one mL of 1 M Sodium hydroxide (NaOH) solution was added, and the final volume of the mixture was brought to 5 mL with distilled water. The mixture was incubated for 15 min at room temperature and absorbance was measured at 510 nm. The total flavonoid content was expressed as quercetin equivalent (µg/mg of extract), which is a common reference standard.

Antioxidant activity

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay

The DPPH radical scavenging activity was carried out according to the method described by Blois[15], where one mL of the carrot peel extracts (ME and AE) were taken in various concentrations and mixed with one mL of 0.1 mM of DPPH solution in methanol. The setup was left at dark in room temperature and the absorption was monitored after 30 minutes. Absorbance was read at 517 nm in spectrophotometer. The ability of the test sample to scavenge DPPH radical was calculated by the following formula:

$$\frac{Absorbance\ in\ control - Absorbance\ in\ sample}{Absorbance\ in control} x\ 100$$

Absorbance control was the absorbance of DPPH and methanol

Absorbance sample was the absorbance of DPPH radical and the test sample.

Ferric Reducing Antioxidant Power assay

The Fe^{3+} reducing power assay was done according to the method described by Yen and Chen[16]. Different

Table 4 a: Phosphomolybdenum assay of carrot peel acetone extract.

S.No	Concentration (µg/µL)	Absorbance at 695 nm
1	50	0.29
2	100	0.63
3	150	0.69
4	200	0.72
5	250	0.73
6	300	0.74

Table 4 b: Phosphomolybdenum assay of carrot peel methanol extract.

S.No	Concentration (µg/µL)	Absorbance at 695 nm
1	20	0.29
2	40	0.74
3	60	0.84
4	80	0.85
5	100	0.86
6	120	0.89

concentrations of the carrot peel AE and ME (each 10 mg) were mixed with one mL of phosphate buffer (0.2 M, pH 6.6) and one mL of potassium ferricyanide (1%). The mixture was incubated at 50ºC for 20 min. Then one mL of 10% trichloroacetic acid was added to the mixture. Followed by addition of one mL of 0.1% of freshly prepared ferric chloride and the absorbance of the resultant solution was measured at 700 nm.

Phosphomolybdenum reduction assay

The antioxidant activity was evaluated by reduction assay method[17] by the formation of green phosphomolybdenum complex. The carrot peel extracts ME and AE (each I mL) of various concentrations were combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. The samples were cooled to room temperature and the absorbance of the mixture was measured at 695 nm against blank.

Antibacterial activity

The antibacterial activity of the carrot peel was analysed by well diffusion method[18]. Muller Hinton agar was prepared according to the standard procedure and 25 mL was poured into the plates and was allowed to solidify. The standard inoculum suspension was streaked over the surface of the media using sterile cotton swab to ensure the confluent growth of the organism and the plates were allowed to dry for 5 minutes. After drying, the different concentrations (50, 75 and 100 µg/mL) of the methanol and acetone extract of carrot peel were poured into the wells. Tetracycline was used as a standard (1 µg/mL) and methanol was used as control. Finally the inoculated plates were incubated for 24 hours at 37ºC. The zone of inhibition was measured and noted.

RESULTS AND DISCUSSION

Total Phenolic and flavonoid content

The total phenolic and flavonoid content of carrot peel extracts (ME and AE) is presented in Table.1. Total Phenolic content and Flavonoid content was higher in carrot peel acetone extract (AE) i.e. 106.36 µg/g of GAE and 27.83 µg/g of QE respectively than methanol extract (ME). Phenolic content can be used as an important indicator of antioxidant capacity and can be used as a preliminary screen for any product when intended to be used as a natural source of antioxidants in functional foods[19]. Phenolic compounds are dietary antioxidants found in plants that are shown to inhibit LDL oxidation, inhibit platelet aggregation and adhesion, decrease total and LDL cholesterol and induce endothelium- dependent vaso-relaxation[20,21,22]. Flavonoids are the major phenolic compounds in plants[23]. They are considered as potential antioxidants exerting their antioxidant activity by the mechanisms of radical scavenging and metal ion chelation to inhibit lipid peroxidation[24].

Antioxidant activity

The antioxidant activity of the carrot peel was evaluated using three *in vitro* assays namely DPPH Assay, FRAP Assay, Phosphomolybdenum Assay.

DPPH Assay

DPPH assay is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity[25]. The free radical scavenging activity was high in acetone extract compared to methanol extract. The result of the DPPH assay of carrot peel is presented in Table 2 a and Table 2 b The AE showed highest activity (96.95 %) at 300 µL concentration while the highest activity for ME i.e. 34.91% was at 600 µL. The IC_{50} carrot peel AE and ME are76 µg/µL and 859 µg/µL respectively. The IC_{50} value of ascorbic acid was 4.89 µg/µL.

FRAP Assay

The antioxidant capacity of the carrot peel extracts is determined by the ability of the antioxidants in these extracts to reduce ferric to ferrous iron in FRAP reagent.The ferric reducing power of the carrot peel AE and ME at various concentrations is presented in Table 3 . From the Table 3, it is evident that the Methanol extract showed greater ferric reducing capacity than acetone extract.

Nguyen et al.,[26] studied the antioxidant activity of the carrot peel extracted with four solvents (methanol, ethanol, water and hexane). The ferric reducing antioxidant power of carrot peel was 15.31, 8.88, 4.82 and 0.31 mg TE/g dry weight for methanol, ethanol, water and hexane, respectively.

Phosphomolybdenum Assay

The results for phosphomolybdenum assay are presented in Table 4 a and Table 4.2. From the Table 4a and Table 4 b, it can be inferred that Methanol extract had greater activity (0.89) for phosphomolybdenum assay than acetone extract of carrot peel.

The peels of vegetable and fruit could be used as natural antioxidant to preserve foods. Addition of other peels with the carrot peel could enhance its antioxidant properties. In a study carried out by Singh & Immanuel[27], the potential

Table 5: Antimicrobial activity of carrot peel acetone and methanol extract.

S.NO	Extract	Organisms	Standard	Zone of Inhibition		
				50 µL	75 µL	100 µL
1	Acetone	*Shigella flexneri*	25 mm	11 mm	12 mm	13 mm
2		*E coli*	22 mm	12 mm	14 mm	16 mm
3		*Staphylococcus aureus*	23 mm	12 mm	13 mm	16 mm
4		*Klebsiella pneumonia*	26 mm	-	13 mm	14 mm
1	Methanol	*Shigella flexneri*	27 mm	14 mm	15 mm	16 mm
2		*E coli*	25 mm	13 mm	14 mm	16 mm
3		*Staphylococcus aureus*	29 mm	12 mm	13 mm	17 mm
4		*Klebsiella pneumonia*	21 mm	12 mm	14 mm	15 mm

of pomegranate, lemon and orange peels as natural antioxidants in paneer was studied. Among the peel used Pomegranate showed greater antioxidant activity by preventing peroxide formation and extending shelf life, followed by lemon peel and orange peel.

Antimicrobial activity

Antimicrobial potential of carrot peel extracts against four pathogens were assessed in terms of zone of inhibition of bacterial growth. The results of the antimicrobial activity are presented in Table 5.

The antimicrobial activity of the extracts increased linearly with increase in concentration of the extracts (µg/mL). The growth inhibition zone measured for acetone extracts for various bacteria ranged from 11-16 mm. The growth inhibition zone measured for methanol extracts were in the range 12-17 mm. The antibacterial potential was dose-dependent against *Shigella flexneri*, *E coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Carrot peel inhibited the growth of four food borne disease causing pathogens. There was little difference in antimicrobial activity in both the extracts. In a study carried out by Al-Baarri[28] showed that addition of carrot extract with Hyphothiocyanite enhanced its antimicrobial activity against *Staphylococcus aureus* and *E coli.*

CONCLUSION

This is a preliminary study to evaluate the antioxidant and antimicrobial potential of carrot peel. Further studies in carrot peel are warranted to validate its functional and physiological benefits. Increasing awareness among consumers about the side effects of synthetic compounds in food and medicine are prompting food industries, scientists and pharmacists to search for newer and natural ingredients for human consumption which are safe and health promoting.

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