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Research Article

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Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts

Moragot Chatatikun[1] and Anchalee Chiabchalard[2*]

[1]*Graduate program in Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Center for Excellence in OMICs Nano-Medical Technology Development project, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand*

ABSTRACT

Daucus carota Linn., commonly known as carrot, is a vegetable which has beta-carotene content. Roots of Daucus carota Linn.have been used for treatments of inflammation and some chronic diseases. However, active compounds in carrot roots that makes these treatments effective remain unknown. The major focus of this study was to determinetotal phenolic, total flavonoid contents and antioxidant activities in petroleum and ethanolic extracts from baby carrot and carrot. Baby carrot and carrot root crude extracts were assayed for total phenolic and total flavonoids contents, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and radical scavenging activity. Ethanolic extracts from baby carrots and carrots showed total phenolic content of 35.9±4.0 and 30.7±3.1 mg GAE/g dry plant material respectively. No phenolic contents were detected in petroleum extracts. Total flavonoid content of ethanolic extracts from baby carrot and carrot were 35.3±6.8 and 20.4±2.8 mg QE/g dry plant material, respectively. Petroleum extracts of baby carrot and carrot had total flavonoid contents of 17.7±2.7 and 3.7±0.7 mg QE/g dry plant material, respectively. Interestingly, ethanolic extracts of baby carrot and carrot had higher antioxidant activities as compared to those of petroleum extracts as measured by DPPH and ABTS assay. Thesefindings could be further used in future investigations which may lead to a development of new drugs for prevention or treatment of chronic diseases that involve damages caused by free-radicals

Keywords: *Daucus carota* Linn., phytochemical, antioxidant, free-radical

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INTRODUCTION

Free radicals are any atoms which have one or more unpaired electron in the outermost shell [1]. These unpaired electrons are very unstable and can attack adjacent molecules such as lipids, proteins and carbohydrates and induce cellular damage [2]. Free radicals involving oxygen are termed reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide [3-5]. The oxidative damage caused by excess ROS may lead to development of many diseases such as heart diseases, congestive heart failure, hypertension, cerebrovascular accidents, and diabetic complications [6].

Antioxidant is a molecule which can prevent effects of oxidation in tissues and can protect cell damaging from free radicals [7]. Plants are potential sources of natural bioactive compounds such as antioxidants and secondary metabolites or organic compounds that have an important role in plant defensive system. When plants absorb sunlight, they can produce oxygen and secondary metabolites. Phenolic and flavonoid are among the most important groups of secondary metabolites that are found in vegetables and fruits [8, 9]. Flavonoids from vegetables and fruits are highly potent antioxidants which could reduce incidence of chronic inflammatory diseases or cancer [10, 11].

Daucus carota Linn. commonly known as "carrots" belong to Family Apiaceae (Umbellieferae) and are cultivated all over the world as vegetable. There are many different vairties of carrot such as yellow, red, orange, white purple and black. Orange carrot is the most abundant and commonly used as food. Orange carrots can be subdivided into two types including baby carrots (aged 2-3 months) and carrots (aged 12-24 months), depends on the age when they were harvested. *Daucus carota* Linn. has been used for treatment of anti-diarrhea, anti-infection, anti-high blood cholesterol, anti-inflammation, anti-seizure, anti-fungal anti-bacteria and anti-cancer [13-18]. Major active compounds consist of phenolic, phytosterol, triterpene and polyacetylene [13, 17, 19-26]. Until now, we have no information about baby carrot. Whether baby carrots contain similar amounts of antioxidants as compared to full-growth carrots is not known. Previous studies revealed the presence of phenolic, flavonoid, carotenoid and anthocyanin in ethylacetate and methanolic extract of carrot roots during a phytochemical screening [27-32]. Ethanolic (80% ethanol) and water extract of carrots had phenolic compound and medium anti-oxidant activity in beta-carotene bleaching assay [33]. Essential oils can be obtained from carrots by simultaneous steam distillation and pentane extraction (SDE) [34]. But up till now, there is little information about ethanolic (95%) and petroleum extracts from carrot roots. Therefore, the focus of this study was to determine total phenolic and total flavonoid contents and antioxidant activities in petroleum and ethanol extracts of baby carrots and carrots and establish their potential effects on health promotion and disease prevention against free radical mediated oxidative stress.

EXPERIMENTAL SECTION

1. Reagent and chemicals

Folin Ciocalteu's Phenol reagent, sodium carbonate, gallic acid, quercitin, 10%Aluminium chloride, ethanol, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, methanol, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate, beta carotene, chloroform, linoleic acid, butylated hydroxytoluene (BHT) were purchased from Sigma chemical Co. (St. Louis, MO.)

2. Plant materials

Roots of *Daucus carota* Linn. (baby carrots and carrots) were collected from King Thung Luang project at Chiangmai, Thailand in January-February 2012. Specimens were identified and authenticated by Professor Kasin Suvatabhandhu Herbariu, Department of Botany in Faculty of Science Chulalongkorn University, Thailand. The voucher numbers of baby carrots and carrots were 013513 (BCU) and 013589 (BCU), respectively.

3. Preparation of plant extracts

Roots of *Daucus carota* Linn.were washed and cut into thin slices, then dried in hot air oven at 40 ^{o}C for 48 h. Dried root were blended by Waring blender and filtered with sieve No. 16. Ten grams of the dry powder wereextracted with petroleum ether (300 ml) and followed by 95% ethanol (300 ml) in a Soxhlet apparatus. The solvents were removed by using a rotary evaporator. Crude extracts were dissolved in dimethyl sulfoxide (100% (DMSO) and stored at -20^{o}C until used.

4. Phytochemical screening

Total phenolic content

The total phenolic contents in baby carrot and carrot crude extracts were determined by using the Folin-Ciocalteu method described by Chang *et al*. (2002) [35]**.** 50 µl of extracts (1 mg/ml) or standard solution of gallic acid (6.25, 12.5, 25, 50, 100 µg/ml) in distilled water were added to 50 µl of distilled water. Distilled water was used as blank. 50 µl of 10% Follin Cicocalteu's phenol reagent and 50 µl of 1 M sodium carbonate solution were added to the mixture in a 96-well plate. Reactions were incubated for 60 minutes at room temperature and protected from light. The absorbance was measured at 750 nm with a Microplate Reader (Biotek, USA.). Total phenolic contents in baby carrot and carrot were expressed as mg Gallic Acid Equivalents (GAE)per gram of dry plant material. All samples were analyzed in triplicate.

Total flavonoid content

Total flavonoid content in baby carrots and carrots were determined by the aluminium chloride colorimetric assay. 50 µl of extracts (1 mg/ml) or standard solution of quercitin (6.25, 12.5, 25, 50, 100 µg/ml) in 80% ethanol was added to 10 µl of 10% the aluminium chloride solution and followed by 150 µl of 95% ethanol. 80% ethanol was used as reagent blank. 10 µl of 1 M sodium acetate was added to the mixture in a 96 well plate. All reagents were mixed and incubated for 40 minutes at room temperature protected from light. The absorbance was measured at 415 nm with a Microplate Reader (Biotek, USA.). Total flavonoid contents in baby carrots and carrots were expressed as mg Quercitin Equivalents (QE) per gram of dry plant material. All samples were analyzed in triplicates.

5. Free radical scavenging activity

DPPH assay

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was performed as described by Yamasaki *et al*. (1994) [36]. Briefly 20 µl of carrot extracts (1 mg/ml) or standard solution of ascorbic acid (3.125, 6.25, 12.5, 25, 50 µg/ml) in absolute methanol was added to 180 µl of DPPH reagent in 96 well plate. Absolute methanol was used for reagent blank. All reagents were mixed and incubated for 30 minutes at room temperature, protected from light.. The absorbance was measured at 517 nm with a Microplate Reader (Biotek, USA.). Experiments were done in triplicates. The percentages of the DPPH free radical scavenging activity were calculated as follows:

$$\% \text{ Scavenging activity} = 100 \times \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right)$$

The percentages of the DPPH free radical scavenging activity were determined by comparing with free radical scavenging activity of ascorbic acid and expressed as mg vitamin C Equivalent Antioxidant Capacity (VCEAC) per gram of dry plant material.

ABTS assay

The ABTS free radical scavenging activity was performed as described by Re *et al*. (1999) [37]. The 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) or $ABTS^{\bullet+}$ formation was generated by oxidation of ABTS reagent and potassium sulfate. 20 µl of extracts (1 mg/ml) or ascorbic acid standard (3.125, 6.25, 12.5, 25, 50 µg/ml) in absolute ethanol was added to 180 µl of $ABTS^{\bullet+}$ working reagent in a 96-well plate. Absolute ethanol was used as reagent blank. The plate was incubated for 45 minutes at room temperature in a dark condition. The absorbance was measured at 734 nm with a Microplate Reader (Biotek, USA.). Experiments were all done in triplicates.

$$\% \text{ Scavenging activity} = 100 \times \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right)$$

The percentages of the ABTS free radical scavenging activity were determined by comparing with calibration curve of ascorbic acid and expressed as mg vitamin C Equivalent Antioxidant Capacity (VCEAC)/g dry plant material.

7. Statistical analysis

The data expressed as mean±S.E.M. from three independent experiments. Statistic significant differences were determined by student's t-test and p values < 0.05 were considered as significant difference.

RESULTS

To determine antioxidant activities, carrot and baby carrot roots were dried and sequentially extracted with petroleum ether and ethanol in a Soxhlet apparatus. After solvent evaporation, crude extracts were tested for antioxidant activities, using different methods and principles and the results were as following;

Total phenolic content

To determine total phenolic contents in carrots and baby carrots, the amount of total phenolic compounds in baby carrot and carrot were assayed by Folin-Ciocalteu method as described by Chang *et al*. (2002) [35]. We found that ethanolic extracts of baby carrot and carrot had total phenolic content of 35.9±4.0 and 30.7±3.1 mg GAE/g dry plant material, respectively. We could not detect any phenolic compounds in petroleum extract fractions (Table 1). This result suggested that there were more phenolic compounds in ethanolic extract than petroleum extract and more phenolic compounds were found in baby carrots as compared to carrots.

Table 1 Total phenolic contents of baby carrot and carrot

Extracts	Total phenolic contents (mg GAE/g dry plant material)
Baby carrot (petroleum extract)	NA
Baby carrot (ethanolic extract)	35.9±4.0
Carrot (petroleum extract)	NA
Carrot (ethanolic extract)	30.7±3.1

Data was shown as mean±S.E.M. of triplicate experiment. (P value <0.05)
NA = not available

Total flavonoid content

Total flavonoid contents in baby carrots and carrots were determined by the aluminium chloride colorimetric as described by Chang *et al*. (2002) [35]. We found that ethanolic extracts from baby carrots and carrots had total

flavonoid contents of 35.3±6.8 and 20.4±2.8 mg QE/g dry plant material, respectively while petroleum extracts from baby carrot and carrot had total flavonoid content of 17.7±2.7 and 3.7±0.7 mg. As shown in Table 2, our results suggested that baby carrots had higher flavonoid contents than carrots and the ethanolic extracts had higher flavonoid contents than the petroleum extracts.

Table 2 Total flavonoid contents of baby carrot and carrot

Extracts	Total flavonoid contents (mg QE/g dry plant material)
Baby carrot (petroleum extract)	17.7±2.7
Baby carrot (ethanolic extract)	35.3±6.8
Carrot (petroleum extract)	3.7±0.7
Carrot (ethanolic extract)	20.4±2.8

Data was shown as mean±S.E.M. of triplicate experiment. (P value <0.05)

DPPH assay

To determine free radical scavenging activity of baby carrot and carrot, DPPH assays were carried out as described by Yamasaki *et al*. (1994) [36]. Our results showed that ethanolic extracts of baby carrots (33.0±1.1 mg VCEAC/g dry plant material) and carrots (27.5±1.7 mg VCEAC/g dry plant material) had significantly higher antioxidant activities as compared to those found in petroleum extracts. Antioxidant activities of petroleum extracts from baby carrots and carrots were 5.0±0.4 and 4.5±0.9 mg VCEAC/g dry plant material, respectively.

Table 3 Antioxidant activities of baby carrots and carrots as determined by DPPH assays

Extracts	Antioxidant activities by DPPH assay (mg VCEAC/g dry plant material)
Baby carrot (petroleum extract)	5.0±0.4
Baby carrot (ethanolic extract)	33.0±1.1
Carrot (petroleum extract)	4.5±0.9
Carrot (ethanolic extract)	27.5±1.7

Data was shown as mean±S.E.M. of triplicate experiment. (P value <0.05)

ABTS assay

To measure free radical scavenging activity, ABTS were carried out as described by Re *et al*. (1999) [37]. Ethanolic extracts from baby carrots and carrots had antioxidant activities of 42.2±3.9 and 34.5±2.8 mg VCEAC/g dry plant material, respectively. Ethanolic extracts of baby carrots and carrots showed high scavenging activities with IC_{50} = 830 µg/ml and 837.5 µg/ml, respectively. Antioxidant activities of petroleum extract from baby carrots and carrots were 14.7±0.9 and 4.2±0.6 mg VCEAC/g dry plant material, respectively.

Table 4 Antioxidant activities of baby carrots and carrots as determined by ABTS assays

Extracts	Antioxidant activities by ABTS assay (mg VCEAC/g dry plant material)
Baby carrot (petroleum extract)	14.7±0.9
Baby carrot (ethanolic extract)	42.2±3.9
Carrot (petroleum extract)	4.5±1.0
Carrot (ethanolic extract)	34.5±2.8

Data was shown as mean±S.E.M. of triplicate experiment. (P value <0.05)

DISCUSSION

Recently, many medicinal plants have been used as alternative medicine for treatments or preventions of several diseases, including diabetes, hyperlipidemia, cancer and Alzheimer's [38, 39] . Medicinal plants have become very popular because they have very few side effects as compared to synthetic drugs. Phytochemicals compounds are widely studied because they are highly abundance in nature and often used as parts of defense mechanisms in plants. Phenolic and flavonoid compounds are major classes of antioxidant compounds found in plants. Carrot roots had previously been investigated and found to contain both phenolic and flavonoid compounds in methanolic fraction [26, 28-32]. Seed oil extract from black carrot had been reported to contain high amounts of phenolic compounds [30]. Commercially available (orange) carrots (14.5 µg of GA/g) had higher level of total phenols as compared to other varieties of colored carrots (4.4 µg of GA/g) [41]. In this study, roots of orange baby carrots and carrots were extracted with petroleum ether and ethanol by Soxhlet method. Our results demonstrated that the amount of phenolic and flavonoid compounds were higher in ethanolic extracts as compared to those found in petroleum extracts. While we could not detect any phenolic compounds in the petroleum fraction, we could detect flavonoid compounds in both petroleum ether and ethanolic fractions of carrots and baby carrots.. However, these levels of flavonoids in petroleum ether part were lower than those found in the ethanolic extract. In another study, the ethyl

acetate extract of Marrubium peregrinum L. (Lamiaceae) also had higher amount of flavonoid contents as compared to total phenolic contents. These results, were similar our results on flavonoid contents in the baby carrot petroleum extract [42]. Generally, when we measured phenolic compounds, we used Folin–Ciocalteu reagent which usually measured polyphenol compounds while flavonoid compounds were determined by, aluminium chloride which react with C-4 keto group and either the C-3 or C-5 hydroxyl group of favones and favonols. Our results showed that antioxidant levels of baby carrot and carrot ethanolic extracts as determined by DPPH and ABTS assay were higher than those of petroleum extracts. Because antioxidant activities were positively correlated with total phenolic and flavonoid contents, suggesting that ethanolic extracts had more antioxidant activities than petroleum extracts and baby carrot had higher antioxidants activities than carrot. Together, our data suggested that the ethanolic extract of baby carrot had the highest antioxidant activities. However, amounts of phytochemicals found in carrots may vary from harvesting, transportation, and processing [22, 43]. Future studies will examine an in vitro model to determine how active compounds found in baby carrot extracts affect signaling pathways mediating oxidative stress.

CONCLUSION

In summary, our results demonstrated that ethanolic extracts of baby carrot and carrot had high amount of phenolic and flavonoid compounds and had high antioxidant activities as measured by DPPH and ABTS. Baby carrots had higher antioxidant activities than carrots and the ethanolic extracts of carrot and baby carrot showed higher antioxidant activities than petroleum extracts. Fractions that have high phenolic or flavonoid contents also had high antioxidant activities when they were assyaed by DPPH and ABTS. Therefore, carrot is a good candidate for a development of new drugs for preventing cell damage caused by free radical exposures and active ingredients in polar solvent fractions such as ethanolic fractions should be identified in future studies.

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