In-vitro radical scavenging activity of *Daucus carota L.* extracts

Nermeen M. Arafa, Usama I. Aly

Department of Plant Biotechnology, Genetic Engineering and Biotechnology Division, National Research Center, Dokki, Giza, Egypt

Correspondence to Usama I. Aly, Department of Plant Biotechnology, Genetic Engineering and Biotechnology Division, National Research Center, Dokki 12311, Giza, Egypt
e-mail: usamajp@yahoo.com

Received 20 October 2017
Accepted 15 December 2017

Egyptian Pharmaceutical Journal
2018, 17:48–52

Background and objective
Antioxidants play a vital role against the harmful effect caused by oxidative stress. The aim of the current study was to assess the antioxidant potential of methanol extracts of *Daucus carota L*. callus addend with an amino acid precursor, l-phenylalanine, under light and dark conditions.
Materials and methods
Callus cultures of petiole, stem, and root explants of *D. carota L.* in-vitro seedlings were implanted on calli maintenance medium fortified with 500 and 1000 mg/l l-phenylalanine as an amino acid precursor and then were incubated under light and dark conditions. The various prepared concentrations of *D. carota L*. callus crude extracts of petiole, stem, and root explants by maceration with 85% methanol were screened for possible antioxidant activity using 2, 2′-diphenyl 1-picrylhydrazyl radical scavenging test.
Results and conclusion
The present study re-cultured the induced calli from petiole, stem, and root explants of *D. carota L.* on the best selected medium [MS-medium supplemented with 1 mg/l benzylaminopurine (BAP)+2 mg/l naphthaleneaceticacid (NAA)] for callus cultures added with l-phenylalanine. The different concentrations (2, 4, 6, 8, 10, 12, 14, 16, and 18 mg/ml) of the prepared callus cultures extracts of stem, root, and petiole explants of *D. carota L.* have been tested for antioxidant effects. The results revealed that, under dark condition, 12 mg of petiole extract concentration exhibited the least concentration of methanol crude extract recording the greatest antioxidant activity (124.71%) on medium containing 1000 mg/l phenylalanine in comparison with other callus extracts. The results of this investigation revealed that calli of *D. carota L.* petiole explant should be incubated under dark condition, and their methanol crude extract ought to be used at a concentration of 12 mg. Hence, *D. carota L.* extract should be considered as a new source of natural antioxidant.

Keywords:
antioxidant, callus extract, *Daucus carota L.*

Egypt Pharmaceut J 17:48–52

1687-4315

Introduction

Vegetables generally are considered a dietary supplement of high nutritional value; among which, carrot is an important source for human and animal nutrition. Carrot is full of vitamins, sugars, and minerals [1]; moreover, carrot has antibiotic efficiency against several microbial strains [2]. Numerous antioxidant products of plant origin have been recognized as free radical or active oxygen scavengers [3,4]. Lately, attention has increased more than before toward finding natural antioxidants to be included in foods and therapeutic resources to substitute artificial antioxidants, owing to their carcinogenicity [5–7]. Natural antioxidants can improve physical fitness of the human body and impede lipid oxidative rancidity in foodstuff [8,9].

Free radicals contribute to many disorders in human health [10,11]. Nowadays, there has been an interest regarding the therapeutic potentials of antioxidants. Besides, well-known and traditionally used natural antioxidants from vegetables, fruits, spices, and many other plant species have been investigated in the search for novel antioxidants [12,13]. There is still a demand to find information concerning the antioxidant potential of more plant species.

Antioxidant activity using different stable radicals is related to different redox potentials and steric properties of the free radicals antioxidants [14,15]. A number of studies were focused on the link between antioxidant properties of phenolics, as hydrogen-donating free radical scavengers, and their chemical configurations [16].

The current study was performed to assess the antioxidant capacity of various concentrations of

 DOI: 10.4103/epj.epj_40_17

Daucus carota L. callus extract in methanol (per mg/1 ml of 85% methanol solution) addend with amino acid precursor, l-phenylalanine, and to elucidate their antioxidant potential under light and dark conditions to reach the highest value of antioxidant activity.

Materials and methods

Plant material

The seeds of *D. carota L.* were obtained from Vegetables Research Department, Agriculture Research Centre, Ministry of Agriculture, Dokki, Giza, Egypt.

Sterilization and incubation conditions

Seeds were surface sterilized using 70% (v/v) Et-OH for 1 min, followed by 75% Clorox solution (5.25% sodium hypochlorite) with a drop of Tween-20 for 20 min. After thorough washing in sterile distilled water, seeds were cultured onto basal MS-medium [17] supplemented with 0.7% (w/v) agar and 3% (w/v) sucrose for germination. The pH was adjusted to 5.8 before autoclaving at 121°C for 15 min. All cultures were incubated under controlled light regime (16 h photoperiod with fluorescent cool white light tubes, with 8 h dark period) at 25±1°C.

Callus induction and maintenance

Petiole, stem, and root explants were excised from *D. carota L.* in-vitro growing seedlings and cultured on MS-medium supplemented with 1 mg/l benzylaminopurine (BAP)+2 mg/l naphthaleneaceticacid (NAA). Cultures were kept in a growth room under the same conditions used for seed germination for 3 weeks. The initiated calli were subcultured on the same medium for maintenance and callus growth.

Precursor feeding

l-phenylalanine was added to culture medium before autoclaving at different concentrations (500 and 1000 mg/l). The control medium was made without precursor additives. Cultures were divided into two groups: the first was incubated under a 16 h/day photoperiod and the second was maintained in dark.

Extract preparation

Callus cultures (20 g of fresh callus) were extracted using a method of maceration with 85% methanol for 24 h at room temperature according to Nermeen *et al.* [7]. Different concentrations of methanol crude extract for calli of *D. carota L.* were performed as follows: 2, 4, 6, 8, 10, 12, 14, 16, and 18 mg of callus crude extract per ml of 85% methanol solution.

2, 2′-Diphenyl 1-picrylhydrazyl free radical scavenging activity

2, 2′-Diphenyl 1-picrylhydrazyl (DPPH) free radical scavenging activity was performed as described before by Brand-Williams *et al.* [18], with some modifications.

Statistical analysis

All experiments were conducted in triplicate. Data are reported as mean±SD.

Results and discussion

As a result of the obtained investigations from the first part of our previous research [7], 1 mg/l BAP+2 mg/l NAA was selected to be the best combination of growth regulators to culture stem, root, and petiole explants of *D. carota L.* for callus induction with considerate addition of phenylalanine at 500 or 1000 mg/l concentrations in the cultivation medium. In this part of our present study, calli derived from stem, root, and petiole explants of *D. carota L.* in-vitro seedlings were extracted by 85% methanol in different concentrations for crude extracts.

Antioxidant activity of different *Daucus carota L.* extracts grown under light conditions

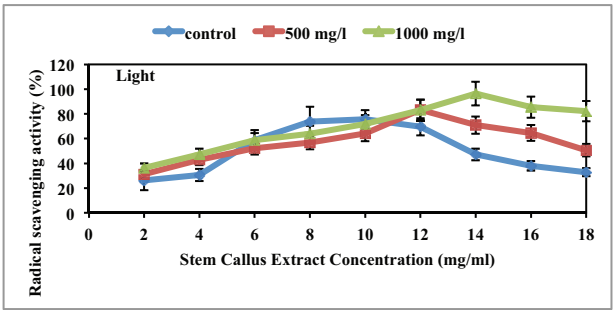
Callus cultures were developed on MS-medium addend with 1 mg/l BAP+2 mg/l NAA (control), phenylalanine (500 or 1000 mg/l) and methanol extracts of source callus at different concentrations (2, 4, 6, 8, 10, 12, 14, 16 and 18 mg). The antioxidant activities were evaluated as free radical DPPH scavenging. This design was conducted throughout the experiment.

Stem callus extracts

The antioxidant activities of stem callus cultures were developed on MS-medium supplemented with 1 mg/l BAP+2 mg/l NAA (control) and fortified with either of 500 or 1000 mg/l phenylalanine, and stem callus extracts at different concentrations (2, 4, 6, 8, 10, 12, 14, 16 and 18 mg) were evaluated as free radical DPPH scavenging, and their results were presented in Fig. 1.

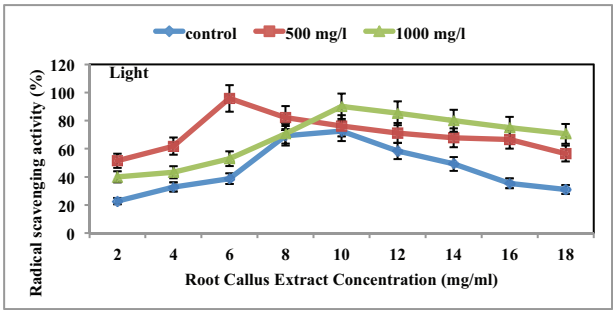
At control treatment, the free radical DPPH scavenging activity of Stem callus extracts (SCE) increased partially from 2 to 8 mg and reached maximum value at 10 mg (75.61%) and then gradually decreased. Alternatively, the highest scavenging activity (96.36%) was detected with 1000 mg/l phenylalanine followed by 83.30% with 500 mg/l phenylalanine at 14 and 12 mg of SCE, respectively (Fig. 1).

Figure 1



Free radical scavenging activity of stem callus extracts of *Daucus carota L.* grown under light condition on MS-medium fortified with phenylalanine (500 and 1000 mg/l) by 1,1-diphenyl-2-picrylhydrazyl radicals. Data are represented as mean±SD of three replicates.

Figure 2



Free radical scavenging activity of root callus extracts of *Daucus carota L.* grown under light condition on MS-medium fortified with phenylalanine (500 and 1000 mg/l) by 1,1-diphenyl-2-picrylhydrazyl radicals. Data are represented as mean±SD of three replicates.

Root callus extracts

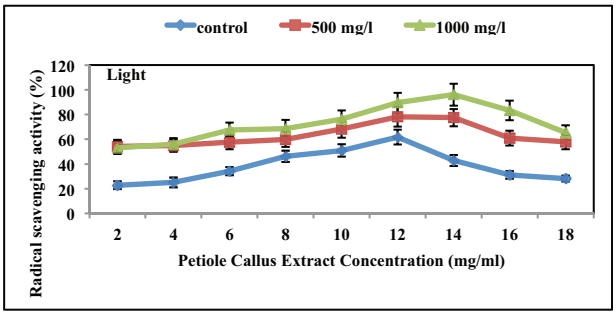
Data in Fig. 2 illustrates that the highest scavenging activity of 95.86% was recorded with root callus grown on MS-medium addend with 500 mg/l phenylalanine, followed by 90.19% with 1000 mg/l phenylalanine at 6 and 10 mg of root callus extracts (RCE), respectively. The minimum scavenging activity (72.68%) was recorded in the absence of phenylalanine at 10 mg of RCE (Fig. 2).

Petiole callus extracts

It is clear that the highest scavenging activity of 96.02% was found with petiole callus grown on MS-medium addend with 1000 mg/l phenylalanine followed by 78.12% with 500 mg/l phenylalanine at 14 and 12 mg of petiole callus extracts (PCE), respectively (Fig. 3). Moreover, at control phenylalanine, the antioxidant activities go down and recorded of 61.79% at and 12 mg of PCE (Fig. 3). It is advisable from data in Figs. 1–3 that using 14 mg concentration of the extracts from stem and petiole calli grown on medium containing 1000 mg/l phenylalanine shows enhancement of their antioxidant efficiency, whereas only 6 mg concentration of the extract from root callus grown on medium modified with 500 mg/l phenylalanine shows so.

Phenylalanine addition has been reported to improve the production of plant secondary metabolite in cell cultures [7]. Phenolics are an important plant product that is mainly derivative from phenylalanine through the phenylpropanoid metabolism [19,20]. Phenylalanine affects plant cell growth and secondary metabolite accumulation in many plant species [21–23]. The authors believed that addition of phenylalanine precursor will increases the metabolic flux during biosynthetic pathway.

Figure 3



Free radical scavenging activity of petiole callus extracts of *Daucus carota L.* grown under light condition on MS-medium fortified with phenylalanine (500 and 1000 mg/l) by 1,1-diphenyl-2-picrylhydrazyl radicals. Data are represented as mean±SD of three replicates.

Many carrot species with appreciable levels of antioxidant activity against the DPPH radical were recognized as potential sources of scavenging compounds [24,7]. Moreover, it is important to mention that light plays an important role in the accumulation of numerous plant secondary metabolites [25–27].

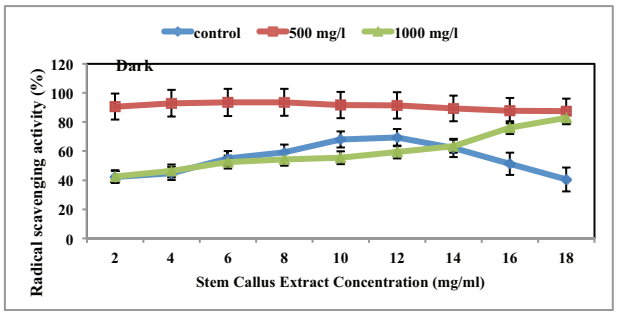
Antioxidant activity of different *Daucus carota L.* extracts grown under dark conditions

Callus cultures were grown on MS-medium supplemented with 1 mg/l BAP+2 mg/l NAA (control), phenylalanine (500 or 1000 mg/l) and methanol extracts of source callus at different concentrations (2, 4, 6, 8, 10, 12, 14, 16, and 18 mg). The antioxidant activities were evaluated as free radical DPPH scavenging. This design was conducted throughout the experiment.

Stem callus extract

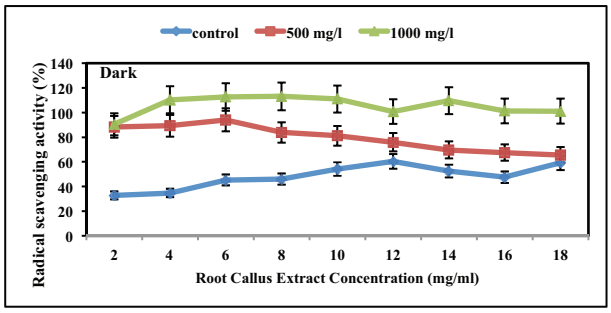
Data in Fig. 4 show that the highest scavenging activity of 93.43% was found with stem callus grown on MS-medium addend with 500 mg/l phenylalanine at 6 mg concentration of SCE followed by 82.88% with

Figure 4



Free radical scavenging activity of stem callus extracts of *Daucus carota L.* grown under dark condition on MS-medium fortified with phenylalanine (500 and 1000 mg/l) by 1,1-diphenyl-2-picrylhydrazyl radicals. Data are represented as mean±SD of three replicates.

Figure 5



Free radical scavenging activity of root callus extracts of *Daucus carota L.* grown under dark condition on MS-medium fortified with phenylalanine (500 and 1000 mg/l) by 1,1-diphenyl-2-picrylhydrazyl radicals. Data are represented as mean±SD of three replicates.

1000 mg/l phenylalanine at 18 mg concentration of SCE and then 69.32% with free phenylalanine medium at 12 mg concentration.

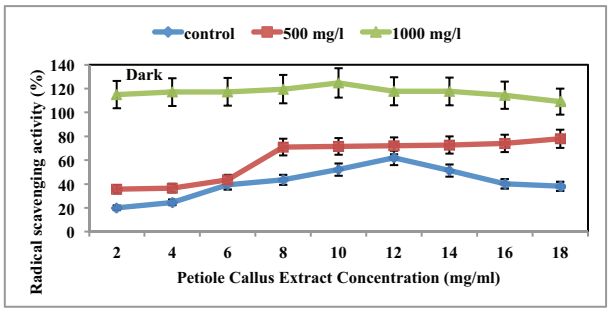
Root callus extract

It is clear that the highest scavenging activity of 113.06% was detected with root callus grown on MS-medium addend with 1000 mg/l phenylalanine followed by 94.06% with 500 mg/l phenylalanine at 8 and 6 mg of RCE, whereas control medium gave 60.32% of scavenging activity at 12 mg concentrations of RCE (Fig. 5).

Petiole callus extract

Petiole callus grown on MS-medium with 1000 mg/l phenylalanine recorded the highest value of antioxidant activity at 124.71%, followed by medium with 500 mg/l phenylalanine (77.97%) and finally phenylalanine-free medium (61.93%) at 12, 18, and 12 mg concentrations, respectively (Fig. 6).

Figure 6



Free radical scavenging activity of petiole callus extracts of *Daucus carota L.* grown under dark condition on MS-medium fortified with phenylalanine (500 and 1000 mg/l) by 1,1-diphenyl-2-picrylhydrazyl radicals. Data are represented as mean±SD of three replicates.

Crud extract of carrot cultures were confirmed to have a variety of antioxidant molecules and phenolic compound [7].

DPPH was commonly applied to check the free radical scavenging capacity [28]. DPPH has a proton free radical with distinguishing absorption at 517 nm, which declines notably by contact with proton of radical scavengers. Current research seems to validate the view that *D. carota* L. callus extract showed a concentration-dependent scavenging of the DPPH radical, which is perhaps owing to its hydrogen-donating ability. The present findings seem to be consistent with other research [29].

Conclusion

Carrot is a well-liked vegetable owing to its valuable contents. It has also a prosperous supply of antioxidants, which protect human being against much serious disorders.

Briefly, under light condition, 14 mg of stem and petiole extract concentrations of *D. carota* recorded the highest antioxidant activity (96.36 and 96.02%, respectively) on medium containing 1000 mg/l phenylalanine, whereas at 6 mg of root extract concentration, the antioxidant activity was slightly decreased (95.86%) with medium containing 500 mg/l phenylalanine. Conversely, under dark condition, 12 mg of petiole extract concentration exhibited the greatest antioxidant activity (124.71%) followed by 8 mg of root extract (113.06%) on medium containing 1000 mg/l phenylalanine and finally 6 mg of root extract (94.06%) with 500 mg/l phenylalanine. The results of this investigation revealed that *D. carota L.* extracts should be considered as a new source of natural antioxidant, taking into account that the various concentrations of petiole, stem, and root calli crude extract per mg per ml of

85% methanol solvent showed a higher scavenging possibility of suppressing the free radicals of DPPH structure.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Nicolle CG, Simon E, Rock PA, Remesy C. Genetic variability influences carotenoid, vitamin, phenolic, and mineral content in white, yellow, purple, orange, and dark-orange carrot cultivars. J Am Soc Hort Sci 2004; 129:523–529.

2 Nermeen MA, Mohamed SS, Usama IA. In vitro antimicrobial activity of carrot callus extracts as affected by tyrosine and tryptophan precursor. Pharm Tech Res 2016; 9:121–129.

3 Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. J Agric Food Chem 1994; 42:629.

4 Duh PD. Antioxidant activity of burdock (*Arctium lappa Linne*): its scavenging effect on free radical and active oxygen. J Am Oil Chem Soc 1998; 75:455–465.

5 Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenecity of buthylated hydroxyanisole in F344 rats. J Nat Cancer Inst 1983; 70: 343–347.

6 Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 2001; 49:5165–5170.

7 Nermeen MA, Mona MI, Usama IA. Evaluation of total phenolic contents and antioxidant activity of carrot callus extracts as affected by phenylalanine precursor. Plant Tissue Cult Biotech 2015; 25:207–221.

8 Pryor WA. The antioxidant nutrient and disease prevention – what do we know and what do we need to find out? Am J Clin Nutr 1991; 53:391–393.

9 Lai LS, Chou ST, Chao WW. Studies on the antioxidative activities of hsian-tsao (*Mesona procumbens Hemsl*) leaf gum. J Agric Food Chem 2001; 49:963–968.

10 Kumpulainen JT, Salonen JT. Natural antioxidants and anticarcinogens in nutrition, health and disease. UK: The Royal Society of Chemistry 1999. 178–187

11 Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afric J Biotech 2006; 11:1142–1145.

12 Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal 2002; 13:8–17.

13 Oke JM, Hamburger MO. Screening of some Nigerian medicinal plants for antioxidant activity using 2,2-diphenyl-picryl-hydrazylradical. Afric J Biomed Res 2002; 5:77–79.

14 Kanungo S, Nahak G, Sahoo SL, Sahu RK. Antioxidant activity and phytochemical evaluation of *Plumbago zeylanica Linn*. *in vivo* and *in vitro*. Int J Pharm Pharm Sci 2012; 4:522–526.

15 Kancko T, Tahara S, Matsu M. Retarding effect of dietary restriction on the accumulation of 8-hydroxy-2-deoxyguanosine in organs of Fisher 344 rats during aging. J Free Radic Biol Med 1996; 23:76–80.

16 Khanam S, Shivprasad HN, Kshama D. *In vitro* antioxidant screening models: a review. Indian J Pharm Educ 2004; 38:180–185.

17 Murashige T, Skoog FA. Revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 1962; 15:473–497.

18 Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol 1995; 28:25–30.

19 Mercier JJ, Arul J, Julien C. Effect of food preparation on the isocoumarin 6-methoxymellein content of UV-treated carrots. Food Res Int 1994; 27:401–404.

20 Dixon RA, Paiva NL. Stress induced phenolpropanoid metabolism. Plant Cell 1995; 7:1085–1097.

21 Shinde AN, Malpathak N, Fulzele DP. Enhanced production of phytoestrogenic isoflavones from hairy root cultures of *Psoralea corylifolia L.* using elicitation and precursor feeding. Biotechnol Bioprocess Eng 2009; 14:288–294.

22 Ouyang J, Wang XD, Zhao B, Wang YC. Enhanced production of phenylethanoid glycosides by precursor feeding to cell culture of *Cistanche deserticola*. Process Biochem 2005; 40:3480–3484.

23 Masoumian M, Arbakariya A, Syahida A, Maziah M. Effect of precursors on flavonoid production by *Hydrocotyle bonariensis* callus tissues. Afr J Biotech 2011; 10:6021–6029.

24 Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004; 85:231–237.

25 Lee WL, Huang JZ, Chen LC, Tsai CC, Chen FC. Developmental and LED light source modulation of carotenogenic gene expression in Oncidium gower ramsey flowers. Plant Mol Biol Rep 2013; 31:1433–1445.

26 Samuolienė G, Brazaitytė A, Jankauskienė J, Viršilė A, Sirtautas R, Novičkova A, *et al.* LED irradiance level affects growth and nutritional quality of Brassica microgreens. Cent Eur J Biol 2013; 8:1241–1249.

27 Wei Z, Luo M, Zhao C, Li C, Gu C, Wang W, *et al.* UV-induced changes of active components and antioxidant activity in postharvest pigeon pea [*Cajanus cajan* (L.) Millsp.] leaves. J Agric Food Chem 2013; 61:1165–1171.

28 Hatano T, Takagi M, Ito H, Yoshida T. Phenolic constituents of liquorice. VII. A new calcone with a potent radical scavenging activity and accompanying phenolics. Chem Pharm Bull (Tokyo) 1997; 45:1485–1492.

29 Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1,-diphenyl-2-picrylhydrazyl. Biosci Biotechnol Biochem 1998; 62:1201–1204.