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ESSENTIAL OIL FROM THE BLACK CARROT LEAVES AND THEIR FREE RADICAL SCAVENING ACTIVITY

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ABSTRACT

Daucus carota L. (black carrot L.) leaves were subjected to hydro-distillation through Clevenger type apparatus for essential oil extraction. Extracted essential oil was separated from water and dried with anhydrous Na_2SO_4. Yeild of the essential oil was 0.02%. Total phenolic content of essential oil determined by using Folin-Ciocalteu reagent taking Gallic acid as standard and measuring at 765 nm by using UV-Vis spectrophotometer. The total phenolic content in carrot leaves were found 8.3 mg GAE/g. The antioxidant activity of essential oil by the DPPH at concentrations 25µL, 50µL, 75 µL and 100 µL was 28%, 38%, 43% and 57%respectively. while 100µL concentration of ascorbic acid give 96%. Antioxidant activity of essential oil of daucus carota is moderate and it was found concentration dependent.

KEYWORDS: Black Carrot leaves, hydro-distillation, Essential oil, Phenolic content, Antioxidant activity.

INTRODUCTION

Black carrots or purple carrots (Daucus carota ssp. sativus var. atrorubens Alef) are characterized by their dark purple- to black-colored roots, due to their high anthocyanin contents. Fruits and vegetables are rich sources of nutrients that contain phytochemicals (also known as bioactive compounds), which are recognised for their nutraceutical effects and health benefits. The cultivated carrot (*Daucus carota* L.) is one of the most important vegetable plants in the world because of its high yield potential and use as fresh or processed

product. With an annual world production (carrots and turnips) of >428 million tons and a total growing area of about 11.5 million hectares, carrots rank among the top 10 vegetable crops in the world.[1] They play a major role in human nutrition, because of their high dietary value and good storage attributes. Phytochemicals contribute to the dietary value of carrots and comprise mainly four types; namely, phenolic compounds, carotenoids, polyacetylenes, and ascorbic acid. Carrot leaves are very rich in several minerals and vitamin such as Na, P, K, Ca, Mg, Mn, Zn, and Fe and vitaminC, A, B1, B2, B6, E, niacinand folate. In D. carota leaf carotenoids are present and beta-carotene which the bodyconverts into vitamin.[4,5] Due the presence of carotenoids in D.carotaleaf, which is useful for diseases of the retina, in skin maintenance and also prevent cancer.[2]

Essential oils, which is present in carrot leaves and used as complementary food in the human nutrition, anticancer, antiviral and also in pharmaceutical industry and in medicine and flavor liqueurs and perfumes.[4,7,8]Essential oil content was higher in carrot leaves (Daucus carota L. subsp. carota (L.) Thel., Apiaceae) than the roots . Essential oils are volatile substances. It contains a chemical component of monoterpenes, diterpenes, sesquiterpenesand oxygenated hydrocarbons derivatives such as aldehydes, ketones, esters and alcohols. The natural source of antioxidants is herbs and spices. Which is used to scavenging free radical to stop their generation.[3,4]

In previous scientific studies noted significant differences in essential oil chemical composition of wild carrots from different regions. Component composition of seeds essential oil of wild carrots depends on the geographical origin of the taxon. Differences in the composition of essential oil of plants may be due to many factors such as differences in the environmental conditions of the region, season of the collection, the development stage and used extract part of the plant and the method of extraction. In folk medicine, the fruits are used as anthelmintic and diuretic, at nephrolithiasis and flatulence. Whole or parts of wild carrot possess antibacterial, diuretic, choleretic and salt soluble effect[2] Depending on the chemical composition of the essential oil exhibits antioxidant, cytotoxic, antibacterial and antifungal activities.[5] The most significant activity was observed in the essential oils of ripe and unripe fruits of wild carrots, containing as major components αpinene, sabinene and α-muurolene.[6] It should be noted that was investigated the composition and the biological activity of essential oils from seeds of cultivated species of Daucus carota ssp. sativa which commercially available in Uzbekistan. The essential oil from this taxon, containing as main

components β-bisabolene (80.49%), β-asarone (8.82%) and β-bergamotene (5.51%) exhibited antimicrobial activity against Staphylococcus aureus and Candida albicans.[7] To the best of our knowledge the essential oil composition and antimicrobial activities of wild carrot from our region has not been reported.

Antioxidant properties play also a pivotal role in some of essential oil biological activities, which is justified by the involvement of oxidative stress in pathology. These attributes are due to the inherent ability of some of their components, particularly phenols, to stop or delay the aerobic oxidation of organic matter, although the procedure by which the oil is obtained from the raw material (distillation) limits the content of phenolics in the final matrix because many such compounds are nonvolatile. This is particularly relevant because most common synthetic antioxidants (such as butylated hydroxyanisole (BHA) or butylhydroxytoluene (BHT)) are suspected to be potentially harmful to human health.[8]

Free radicals reactive oxygen species and reactive nitrogen species generated in our bodies can create oxidative stress. A balance between free radicals and antioxidants is necessary for proper physiological function. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping this oxidative stress. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be dangerous for human health.[9]

Leaves of daucus carota have no particular utility and these are being used as feed for animals. Though these leaves are rich in aroma and thus, the current search was designed to investigate the essential oil from back carroty for their antioxidant activity.

MATERIAL AND METHOD

Daucus carota. (black carrot L.) fresh leaves were collected from local market of Kasur in province of Punjab (Pakistan). These were chopped and used for essential oil extraction by using clevenger type hydrod-distillation[22] for 6 hours. The essential oil was extracted and dried over anhydrous sodium sulfate anhydrous.

Total phenolic content

The total phenolic contents in *Daucus carota. Ssp. Sativus. Hoffman. L. were* determined by using the Folin-Ciocalteu method.[23] An aqueous aliquot (0.025ml) of the extracts was added

to 3.975 ml of distilled water in a test tube, followed by 0.25ml of Folin-Ciocalteu reagent. After 3min. 0.75ml of 20% sodium carbonate was added. The blue coloration absorbance was measured at 765nm was read on a UV-Vis spectrophotometer. The distilled water was used as blank.Total phenolic contents in black carrot leave were expressed as mg Gallic Acid Equivalents (GAE) per gram of extract.The standard curve equation is A (absorbance) =0.003x+o.ooo6 (R^2=0.986)

TPC can be calculated as follow .TPC =cV/m

Where, c=concentration from calibration curve

V=volume of sample used.

M=mass of sample used.

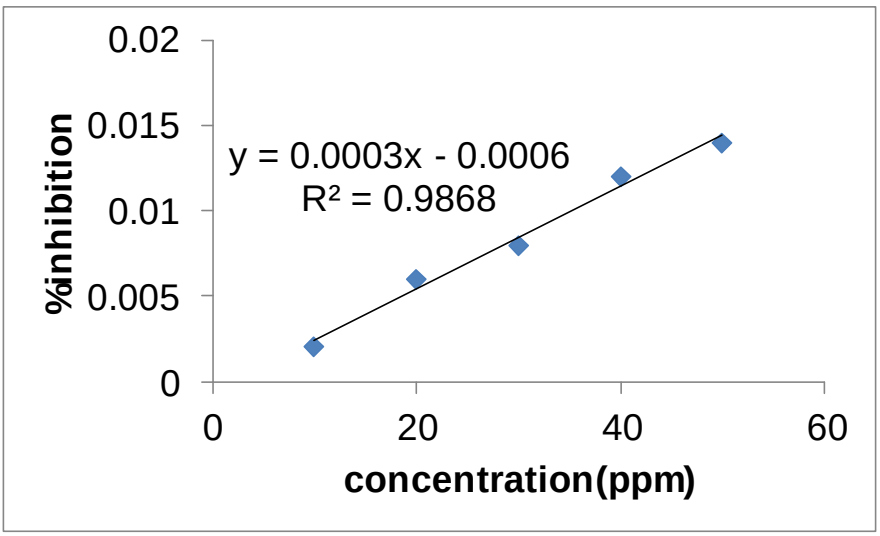

FIG. 1: Standard curve of different concentration of Gallic acid.

DPPH radical scavenging activity

Antioxidant activity of the essential oil of the fresh green leaves of docus carota was evaluated by using 2,2-diphenyl-1-picrylhydrazyle (DPPH) radical. The DPPH assay was performed by following the method of.[11] Briefly, the samples of different concentration of 25 µL, 50 µL, 75 µL, and 100 µL were mixed with 3 ml of methanol of DPPH solution. The absorbance of the resulting solution and the blank (with only DPPH) were recorded at λmax 517 nm by UV-Vis spectrophotometer, after an incubation time of 30 minutes at ambient temperature against ascorbic acid as a positive control. For each sample three replicates were recorded. The percentage of radical scavenging activity was calculated using the following equation.

DPPH scavenging efficiency (%) = (A0-A1)/A0 × 100

Where A0 is the absorption of the control at 30 minutes and A1 is the absorbance of the sample at 30 minutes.

RESULTS AND DISCUSSION

Extraction essential oil from the daucus carota leaves by hydro- distillation process. It was pale in color and 0.02% yield was obtained. The yield of essential oil in literature 2.1% and 0.2% wasstudied[17,25] Major constituents in the leaves of docus carota has studied by Natanzi and Rahmatipour in 2014. They have identified 90 component present in leaves of dacus carota essential oil. These are β-selinene, α-selinene, geranial,γ-cadinene,anethole,Z-methyliso-eugenol, β-asarone, trans-isoeugenol α-pinene (27.44%), sabinene (25.34 %), germacrene D (16.33 %) were present in leaves essential oil.

In this study, quantitative analysis was done by using spectrophotometer for the determination of total phenolic content in essential oil of leaves of *Daucus carota. Ssp. Sativus. Hoffman*. Total phenolic content was calculated by using calibration curve of standard Gallic acid. It results was expressed mg Gallic acid equivalent (GAE/g). The calculated TPC of carrot leaves essential oil was 8.3 mg GAE/g .As reported thatin yellow carrot leaves TPC calculated is 82.07mg/ml(4). In 2016, reported that the total phenolic content are in this order radish leave>yam>sweet potato>carrot leaves. Results show that the total phenolic in the methanol extract of carrot (9.3 mg GAE/g) and sweet potato (17.3 mg GAE/g) leaves (2). Fig.1 shows the calibration curve of Gallic acid at different concentration. The change in total phenolic content is due to geological changes such as environment.

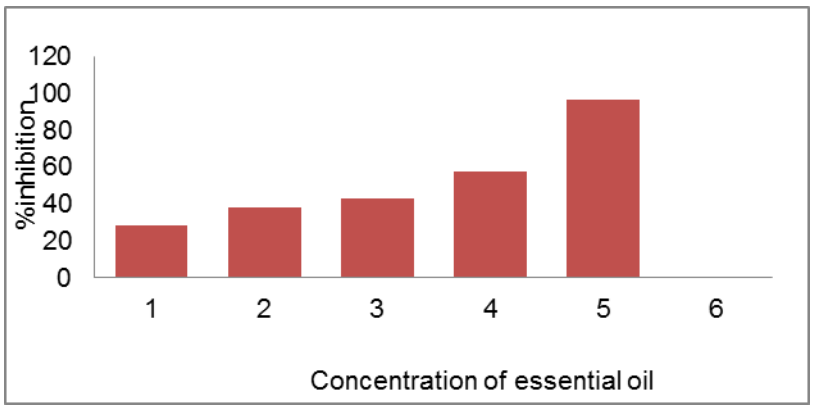

Fig 2: DPPH free radical scavenging activity of essential oil of daucus carota.

In fig. 2 1, 2, 3, 4 and 5 denotes 25 µL, 50 µL, 75 µL, 100 µL for the essential oil from the leaves of daucus carota and 100 µL of ascorbic acid. The antioxidant activity of essential oil

by the DPPH at concentrations 25µL, 50µL, and 75µl was 28%, 38%, 43% respectively. Maximum antioxidant activity 57% was observed at 100 µL, while at same concentration ascorbic acid give 96%. Antioxidant activity of essential oil was found concentration dependent.

The antioxidant potential of essential oil mainly depends on their chemical compositions; particularly phenolic and other secondary metabolites bind with double bonds, which is responsible for the substantial antioxidant activity. Eessential oils extracted from traditional plants such as Achilleafilipendulina, Galagania fragrantissima, Anethum graveolens, A. rutifolia, Hyssopusseravschanicus, Mentha longifolia, and Ziziphorac linopodioidesarerich sources of oxygenated monoterpenes such as aldehydes, ketones, andesters. In addition, monoterpene hydrocarbons (A. absinthiumand A. scoparia) and phenolic terpenoids, such as thymol or carvacrol (O. tyttanthumand Mentha longifolia) are the major chemical compounds, which result in the strongest antioxidant activities. Thymol and carvacrol are important monoterpenes present in several types of EOs extracted from O. tyttanthum, Mentha longifolia and Thymusserpyllus, and they play a key role in the antioxidant properties.[65] The oils extracted from the medicinal plants such as cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme show significant antioxidant activitiesdue to the presence of major constituents such as thymol and carvacrol.[66] Mainly, their activities are related to the presence of phenolic com-pounds that have significant redox properties and play important roles inneutralizing free radicals and in peroxide decomposition.[2] The othercomponents such as certain alcohols, ethers, ketones, aldehydes, andmonoterpenes: linalool, 1,8-Cineole, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes also play a key role inthe antioxidant properties of essential oils.[67]

CONCLUSION

Essential oil was extracted from the leaves of Daucus carota L. (black carrot L.) through Clevenger type apparatus by hydro-distillation. Essential oil was separated from water and dried with anhydrous Na_2SO_4 and 0.02% yield was obtained. The total phenolic content of essential oil determined by using Folin-Ciocalteu reagent taking Gallic acid as standard and measuring at 765 nm by using UV-Vis spectrophotometer. The total phenolic content in carrot leaves were 8.3 mg GAE/g. The antioxidant activity of essential oil by the DPPH at concentrations 25µL, 50µL, and 75µl was 28%, 38%, 43% respectively. Maximum

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