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ESSENTIAL OIL FROM THE ROOTS OF BLACK CARROT (*DAUCUS CAROTA.L. SUBSP SATIVUS HOFFMAN*) AND THEIR ANTIOXIDANT ACTIVITY

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ABSTRACT

Black carrot (daucus carota) belongs to family *Apiaceae (umbelliferae)* and it is primordial important for human nutrition and health. In this study black carrot was collected, cut into small pieces and dried under shade for 20 days. Dried black carrots were subjected to hydro-distillation for essential oil extraction by using *Clevenger type apparatus*. An emulsion was obtained which was separated by using 1-butanol and organic layer containing essential oil was dried by using sodium sulphate anhydrous and then oil was separated by slow evaporation of solvent at ambient conditions. The total phenolic contents of the essential oil were determined by using folin- ciocalteu reagent taking Gallic acid as standard and measuring at 765 nm by using UV-Vis spectrophotometer. Phenolic contents were 11 mg GAE/g. Antioxidant activity at 25, 50 and 75µl was 31, 38 and 46% respectively. Maximum antioxidant activity was 60% observed at concentration 100 µL while at same concentration ascorbic acid give 96.6%. Antioxidant activity of the essential oil was found to be concentration dependent.

KEYWORDS: *Daucus carota*, Hydro-distillation, Essential oil, Total Phenolic Contents, Antioxidant activity.

INTRODUCTION

Carrot (*daucus carota L. Sativus*) is important root vegetable which is belong to the family of *Apiaceae (umbelliferae)* and it is big source of natural antioxidant such as anthocyanins, carotenoids (α, β, ƛ carotene) phenols, vitamin A, B and C. Carrot provide 17% total vitamin A consumption.[1,2,3] Carrot different colored varieties and subspecies are distributed in different part of the world, which are mainly grouped as Western carrot (yellow) Eastern carrot (black).[4] Carrots are found in Europe Afghanistan and adjacent regions of Russia, Iran, India, Pakistan, Anatolia and Africa. Some species are also present in North America and Australia.[5, 6] Carrot extract reported to have anti-nociceptive and anti-inflammatory, hypoglycaemic and antidiabetic activity, antioxidative and anticancer effects.[7] Essential oils are complex mixture of volatile substance such as terpenenes and oxygenated hydrocarbons derivatives such as aldehydes, ketones, esters and alcohols. Essential oils contain about 1,200 compounds such as terpenes plus their corresponding aldehydes, phenylpropanoids, alkanones, alcohols, oxides, sulfur, esters.[8, 9] Carrot seed oil is used as a flavoring agent in food products and perfumery because it blends very well in all kinds of perfumes.[10] Most of vegetables and fruits are consist of phenolic compound. Active oxygen specie, electrophiles, to inhibit nitrosation, to chelate metal ions is scavenged by them and they show anti-oxidant activity.[11] Flavanoids, tannins, aromatic ring (phenolic acids), mainly chlorogenic acid are phenolic compound which are present in roots, bark, nut and seed of the vegetables and fruits. Total phenolic content of chlorogenic are 42.2-61.8%. Chlorogenic are present in baby carrot root. They contain phenolic hydroxyl group which have redox potential, antioxidant activity depend on it and act as reducing agents, hydrogen donators, and singlet oxygen quenchers.[12, 13] Purple carrots contain at least 40 phenolic compound chlorogenic and caffeic acids as the prevalent compound.

Anthocyanins belong to the subclass of Flavonoids and responsible of intense color in fruits vegetables and flower. Anthocyanins and chlorogenic are natural antioxidant in purple carrot and have ability to stop free radical reaction occur in human body.[14, 15] Anthocyanins in nature are derivatives of six common backbone structure that are glycosylated and glcosylations which can form linkage with aromatic acids, aliphatic acids and methyl ester.[16] Many plant species flowers and fruit contain anthocyanins due to its low toxicity it is use as high potential food colorant. Black color of carrots is due to the presences of anthocyanins in tissues and cell cultures.[17] Different factors such as pH, temperature, light, metallic ions and the presence of enzymes can change the stability of anthocyanins.[18]

Carrot seed oil and leaves essential oil has been studied by various authors but from the best of our knowledge this is the first report to study the essential oil of roots and its antioxidant activity. Thus, the current research was designed to investigate the essential oil of roots of black carrot for their total phenolic content and antioxidant activity. The objective of this study is to evaluate anti-oxidant activity and total phenolic content from the essential oil of black carrot.

MATERIAL AND METHODS

Plant material

Black carrot (*daucus carota L. ssp. Sativus Hoffman*) roots were collected from kasur (Pakistan) in December 2018 for extraction of essential oil. Hairs of carrot were removed then wash with water to remove soil and dirt, cut carrot into small pieces about 1-2 cm and dried under shade for 20 days. As shown in fig.1.

Fig.1: black carrot roots use for experiment.

Chemicals

Methanol (MERCK); 1-butanol (MERCK); Distilled water; sodium sulphate (sigma chemical co.); folin-ciocalteu (sigma chemical co.); sodium carbonate (sigma chemical co.); DPPH (2,2-diphenyl-1-picryl hydrazyl) (sigma chemical co.); Gallic acid (MERCK); Ascorbic acid. All the chemicals used including solvent were of analytical grade.

Extraction of essential oil

Dried (10Kg) black carrots were subjected to hydro-distillation for essential oil extraction by using *Clevenger type apparatus* heating 6h. An emulsion was obtained which was separated by using n-butane in a separate vial. Organic layer containing essential oil was dried by using sodium sulphate anhydrous and then oil was separated by slow evaporation of solvent at ambient conditions, stored at 4 °C in the fridge until the time of examination.

ANALYSIS OF ESSENTIAL OIL

Determination of total phenolic content

Stock solution of Gallic acid was prepared with concentration of 1M (1g/L) by using methanol as solvent. Then dilution of 10, 20, 30, 40 and 50ppm were prepared from stock solution for preparation of calibration curve. Total phenolic content of the essential oil of *daucus carota* was evaluated by folin-ciocalteu method [19]. 0.025ml essential oil was added to 3.975ml of distilled water in test tube. Then add 0.25ml *folin-ciocalteu* reagent. After 3 min, 0.75ml of 20% sodium carbonate was added tube content were heated for 15 minutes. The blue colouration was read at 765nm using UV/VIS spectrophotometer against blank, i.e. distilled water. Result was expressed in mg of Gallic acid/g of sample. The standard curve equation is A (absorbance) = 0.0003x+0.0006 (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

Antioxidant activity of essential oil of *daucus carota* root

Antioxidant activity of essential oil of *daucus carota* was evaluated by using 2, 2 - diphenyl-1-picrylhydrazyl (DPPH) radical using ascorbic acid as standard. It was performed by following the method of [20]. 0.4g of DPPH was dissolved in 100ml of methanol to make 0.04% solution of DPPH. Samples of different concentration of 25, 50, 75 and 100 ul 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 λ 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 samples three replicates were recorded. The percentage of radical scavenging activity was calculated using the following equation.

DPPH scavenging effect (%) = $(A_0-A_1)/A_0 \times 100$

Where A_0 is the absorption of the control at 30 minutes and A_1 is the absorbance of sample at 30 minutes.

RESULTS AND DISCUSSION

The essential oil from roots of black carrot (*Daucus carota*) was pale in colour and 0.02% yield was obtained. In reviewing literature, the yields of essential oil from different locations

were 0.033, 0.084, 0.012, 0.223, 0.280, 0.200 and 0.298% [21]. Result reported previously in literature shown that essential oil of root contains Myristicine (29.7%), dillapiole (46.6%), α-farnesene (17.1%), caryophyllene (10.9%), α-Terpinolene (26-56%) other components are α-pinene, humulene and bornyl acetate [22, 23, 24, 25 and 26]. Variations in yield are due to extraction method use, the plant species itself, the plant organ, the stage, harvest time, geographical origin, climatic environmental factor (temperature and humidity) and conservation of plant material.[27]

Total phenolic content was calculated by using calibration curve of standard Gallic acid and results were expressed as mg Gallic acid equivalent (mgGAE/g). They were 11 mg GAE/g. As reported earlier the TPC of purple carrot was 102±3.8 mg/100g.[32] Another researcher shows differences in the content of total phenolics among different coloured carrot varieties (orange, purple, yellow and white) that the TPC of purple carrots was 74.6 mg/100g.[3] The difference in phenolic acid composition is due to differ between cultivators, as well as the part of plant use.[28] Figure.2 shows the calibration curve of Gallic acid at different concentration. The change in total phenolic content is due to geological changes such as environment, soil growing condition and cultivation season. Free flavones are responsible in increase of total phenolic content.[29]

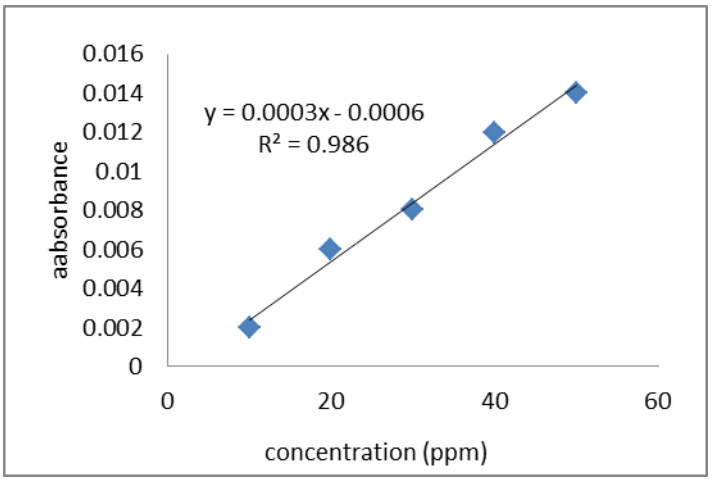


Fig. 2: shows the calibration curve of Gallic acid at different concentration.

The radical scavenging activity of antioxidant is estimated by using DPPH free radical which is stable radical. In the DPPH test, the antioxidant was able to reduce the stable DPPH radical violet colored to the yellow colored diphenyl picryl hydrazine. The effect of antioxidant on DPPH radical scavenging was catch on to their hydrogen-donating ability.

Antioxidant activity of essential of *daucus carota* was measured by using DPPH at 100µL was 60% as compared to the standard anti-oxidant ascorbic acid whose inhibition was 96.67%.

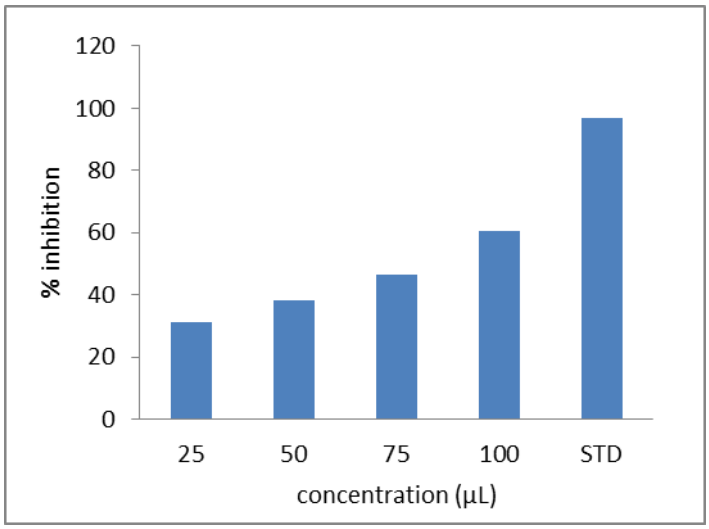
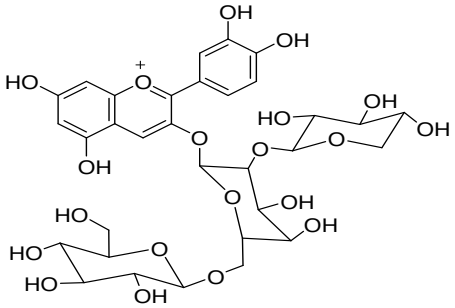


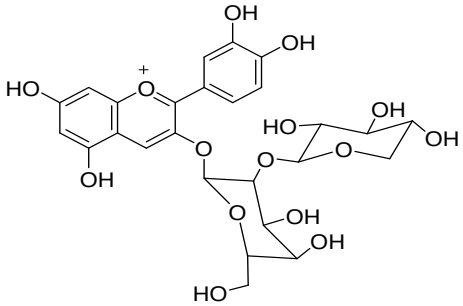
Fig. 3: DPPH free radical scavenging activity of essential oil of daucus carota root.

Fig.3 shows the % inhibition of essential oil at different concentration. Antiradical activity of purple carrot root extract was about 50% by using DPPH radical as previously reported.[12] Presence of carotenoids, polyphenols, polyacetylene, ascorbic acid and vitamins in carrots act as antioxidant, anti-carcinogens and immunoenhancer. They neutralize the free radical and reduce the risk of cancer.[30, 31] The result indicates that essential oil of daucus carota is able to reduce the stable free radical DPPH to yellow DPPH.H moderately.

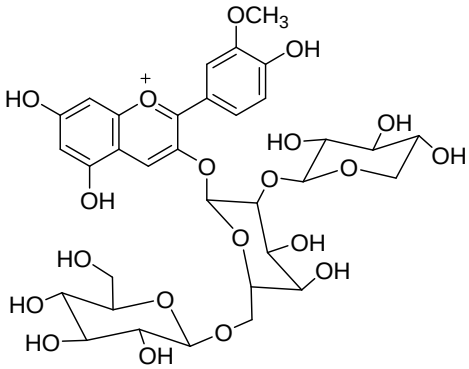
Anthocyanins are present in black carrot increase the interest of researcher because they are use as artificial colorant in food and pharmaceutical industries. It is reported that purple carrots contain 168.70 mg/100g on fresh weight of anthocyanins pigment.[32, 33] Another researcher show anthocyanins in black carrot extract with acetone and ethanol were 344.5 mg/100g and 270.3 mg/100g respectively.[34] Black carrot contain high anthocyanin content that is (1750 mg/kg) black carrot are resistant to PH then other plants.[35] Anthocyanin content can be finding according to the PH differential method and expressed in mg of cyaniding-3-glucoside equivalent per100g dry weight.[36]



1) Cyanidin 3-xylosylglucosylgalactoside

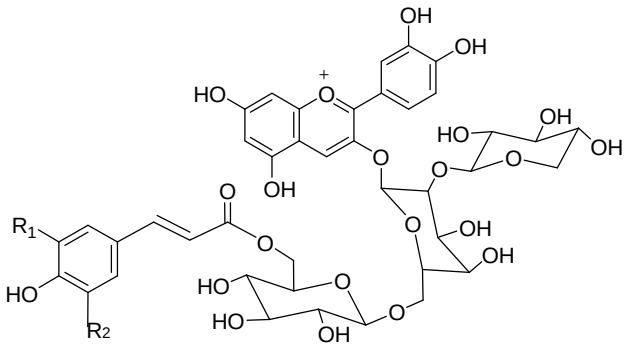


2) Cyanidin 3-xylosylgalactoside



3) Peonidin 3-xylosylglucosylgalactoside.

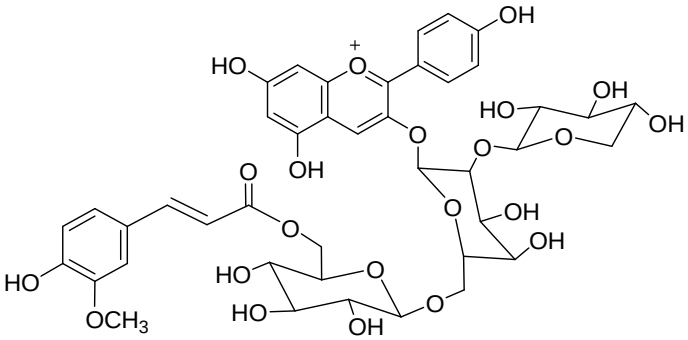
Fig. 4: chemical structure of non-acylated anthocyanins detected in black carrot extracts: (1) Cyanidin 3-xylosylglucosylgalactoside, (2) Cyanidin 3-xylosylgalactoside, (3) Peonidin 3-xylosylglucosylgalactoside.



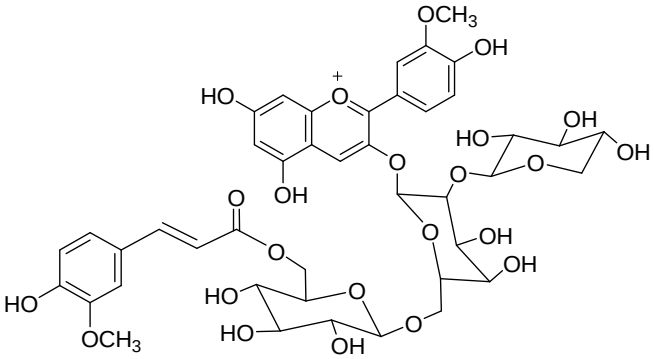
4) R_1=R_2=OCH_3: sinapic acid derivative of cyanidin 3-xylosylglucosylgalactoside

5) R_1=H; R_2=OCH_3: ferulic acid derivative of cyanidin 3-xylosylglucosylgalactoside

6) R_1=R_2=H: coumaric acid derivative of cyanidin 3-xylosylglucosylgalactoside

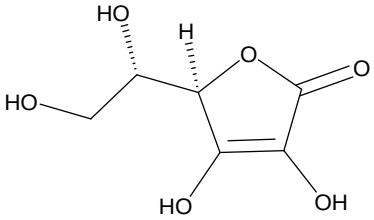


7) Ferulic acid derivative of pelargonidin 3-xylosylglucosylgalactoside

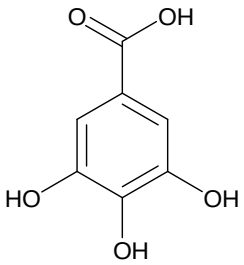


8) Ferulic acid derivative of peonidin 3-xylosylglucosylgalactoside

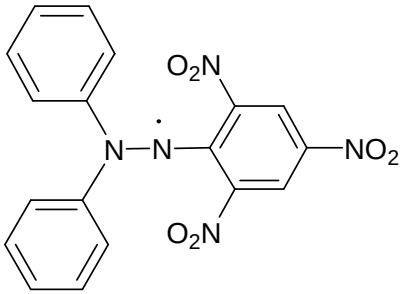
Fig. 5 chemical structure of acylated anthocyanins detected in black carrot extract: (4) sinapic acid derivative of cyanidin 3-xylosylglucosylgalactoside, (5) ferulic acid derivative of cyanidin 3-xylosylglucosylgalactoside,(6) coumaric acid derivative of cyanidin 3 xylosylglucosylgalactoside,(7) Ferulic acid derivative of pelargonidin 3-xylosylglucosylgalactoside,(8) Ferulic acid derivative of peonidin 3-xylosylglucosylgalactoside. Adopt from.[37]



Ascorbic acid



Gallic acid



DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical

Fig. 6: structure of different standards used.

CONCLUSION

Essential oil from the black carrot *(daucus carota)* was extracted by hydro-distillation, its total phenolic content was determined by standard curve of Gallic acid and antioxidant study is carried out with DPPH. Total phenolic content of essential oil were determined (11 GAE/g). Antioxidant activity of essential oil was determined by using DPPH and taking ascorbic acid as standard. Antioxidant activity of essential oil of carrot observed at 100µL was 60% respectively. Moderate antioxidant activity was observed in the essential oil from roots of kanji *(daucus carota).* It is conclude that there is direct relationship between phenolic content and antioxidant activity. Increase in phenolic content increase its antioxidant activity.

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