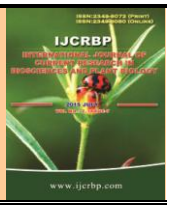
Int. J. Curr. Res. Biosci. Plant Biol. 2015, 2(7): 168-172

EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 7 (July-2015) pp. 168-172

www.ijcrbp.com



Original Research Article

Phytochemical Screening, Characterization, Compound Identification and Separation from *Daucus carota* L.

S. Sivanantham* and N. Thangaraj

P.G and Research Department of Botany, Kandaswami Kandars College, Velur, Namakkal - 638 182, Tamil Nadu, India

**Corresponding author.*

A b s t r a c t

The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline some where in between natural products (organic chemistry) and plant biochemistry and is related to both. The present study deals with the isolation and partial purification of bioactive compounds from the crude ethanol extracts of the vegetable *Daucus carota* L. (Apiaceae). The identification and isolation of bioactive compounds in the crude extract and active bands isolated by preparative column chromatography and thni layer chromatography were accomplished using characterization analysis. Identification of unknown compound was established through comparison of spectral data (UV, IR) with literature values. The preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, carbohydrate, phenol, flavonoids, coumarin and chlorogenic acid. This study confirms that terpenoid is an active component in the present investigation.

K e y w o r d s

Antioxidant compound

Daucus carota

Ethanolic extract

Terpenoid

Introduction

A number of medicinal plants abound in Nigeria's flora (Gbile, 1986) which is the richest country in West Africa with regards to medicinal plant resources. The country exhibits a wide range in terms of climate and topology which has a bearing on its vegetation and floristic composition. Herbal preparations are used in traditional medicine as crude drugs in various dosage forms, as whole, crushed, powdered forms, decoctions, dried extracts, infusions, poultices and tinctures (GHP, 2007). Many of these plants have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of organisms (Fraenkel, 1959). Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group (Stamp, 2003). Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses.

The terpenoids are large and diverse class of naturally occurring organic chemicals similar to terpenes, derived

from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products.

Scientific classification of *Daucus carota*

Kingdom : Plantae
Order : Apiales
Family : Apiaceae
Genus : *Daucus*
Species : *carota*

The carrot (*Daucus carota* subsp. *sativus*; etymology: from Late Latin carota, from Greek καρωτόν karoton, originally from the Indo-European root ker- (horn), due to its horn-like shape) is a root vegetable, usually orange in colour, though purple, red, white, and yellow varieties exist. It has a crisp texture when fresh. The most commonly eaten part of a carrot is a taproot, although the greens are sometimes eaten as well. It is a domesticated form of the wild carrot *Daucus carota*, native to Europe and southwestern Asia.

The domestic carrot has been selectively bred for its greatly enlarged and more palatable, less woody-textured edible taproot. The Food and Agriculture Organization of the United Nations (FAO) reports that world production of carrots and turnips (these plants are combined by the FAO for reporting purposes) for calendar year 2011 was almost 35.658 million tones. Almost half were grown in China. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are a tradition in many regional cuisines.

Description

The carrot is a variable biennial plant, usually growing up to 1 m tall and flowering from June to August. The umbels are claret-coloured or pale pink before they open, then bright white and rounded when in full flower, measuring 3–7 cm wide with a festoon of bracts beneath; finally, as they turn to seed, they contract and become concave like a bird's nest. The dried umbels detach from the plant, becoming tumbleweeds.

Similar in appearance to the deadly poison hemlock, *Daucus carota* is distinguished by a mix of bi-pinnate and tri-pinnate leaves, fine hairs on its stems and leaves, a root that smells like carrots, and occasionally a single dark red flower in its center.

Chemical constituents

Polyacetylenes can be found in Apiaceae vegetables like carrots where they show cytotoxic activities. Falcarinol and falcarindiol (cis-heptadeca-1, 9-diene-4,6-diyne-3,8-diol) are such compounds (Garrod et al., 1978). This latter compound shows antifungal activity towards *Mycocentrospora acerina* and *Cladosporium cladosporioides*. Falcarindiol is the main compound responsible for bitterness in carrots. Other compounds such as pyrrolidine (present in the leaves), 6-hydroxymellein, 6-methoxymellein, eugenin, 2,4,5-trimethoxybenzaldehyde (gazarin) or (Z)-3-acetoxy-heptadeca-1,9-diene-4,6-diin-8-ol (falcarindiol 3-acetate) can also be found in carrot (Wikipedia, 2015).

Uses

Daucus carota root is edible while young. The leaves of the wild carrot can cause phytophotodermatitis, so caution should also be used when handling the plant. If used as a dyestuff, the flowers give a creamy, off-white color. Folk-medicine holds that an infusion of the seeds will inhibit pregnancy. *Daucus carota*, when freshly cut, will draw or change. Note that this effect is only visible on the "head" or flower of the plant. Carnations also exhibit this effect. This occurrence is a popular science demonstration in primary grade school. The present study deals with the effects of bioactive metabolites presents in the fresh vegetable namely *Daucus carota* and its characteristics - phyto-chemical screening and isolation of bioactive compounds.

Materials and methods

The fresh samples of *Daucus carota* was collected from Local Market in early morning. Then the samples were washed with water freed from extraneous matter and brought to the lab in plastic bags. Ten gram of plant material was crushed and ethanol extracted. The extract was filtered using Whatmann filter paper and concentrated. The extract was put in airtight container and stored in refrigerator which was subjected to following analysis.

The extract was subjected to qualitative test for the identification of various plant constituents by Harborne method (1973) as shown in Table 1.

Table 1. Preliminary phytochemical analysis of the extracts.

Tests	Analytical procedure
Alkaloid	A small quantity of the extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.
Mayers test	0.5ml of extract was treated with few drops of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.
Dragendroff's test	0.5 ml of extract was treated with Dragendroff's reagent (potassium bismuth iodide). Formation of orange or orange red precipitate indicates the presence of alkaloid.
Wagner's test	0.5 ml of extract was treated with few drops of Wager's reagent gives an brown or reddish brown precipitate indicates the presence of alkaloid.
Preparation of Wagner's reagent	Iodine (1.27 g) and potassium iodide (2 g) was dissolved in 5 ml of water and made up to 100 ml with distilled water.
Carbohydrate	0.5 ml of extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to following tests to detect the presence of carbohydrates.
Molish's Test	0.5ml of extract was treated with 1 ml of Alpha napthol & Conc. H_2SO_4, which gives a purple color.
Steroid: Libermann's Burchard Test	0.5ml of extract was treated with few ml of chloroform, acetic acid and conc. H_2SO_4 which gives bluish green color.
Saponins	0.5 ml of extract was treated with 1 ml Conc. H_2SO_4, gives green color indicates the presence of saponins.
Tannins	0.5 ml of sample was treated with lead acetate solution; formation of precipitate indicates the presence of Tannins.
Chlorogenic acid	0.5 ml of sample was treated with few ml of aqueous ammonia and was exposed to air which gradually develops a green color indicates the presence of Chlorogenic acid.
Flavonoids	0.5 ml of sample was allowed in a few ml of ammonia. The mixture was observed under UV and visible lights - formation of fluorescence colour indicates the presence of flavonoids.
Coumarin	0.5 ml of sample was treated with 10% Sodium chloride, formation of yellow colour indicates the presence of Coumarin.
Flavones	0.5 ml of sample was treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.
Anthocyanin	0.5 ml of sample was treated with aqueous sodium hydroxide indicates the presence of anthocyanin with the formation of blue violet color.
Terpenoids	5ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for presence of terpenoids.
Phenol	0.5ml of extract was treated with 1 ml phenol reagent was added and wait for few minutes formation of blue color indicates the presence of phenol.

Isolation of bioactive compounds by Column Chromatography (Rangari, 2008)

The bottom of the column was first plugged with little glass wool and then clean sand bed was placed over the glass wool. The sand bed serves to give a flat base to the column of the adsorbent. Then the dried Silica Gel 100-200 mesh was poured into the column. After 2/3rd of the column was filled with the powder, it was tabbed, and set aside. After that, a filter paper disc and sand bed were placed over the adsorbent in order to avoid the disturbance of the adsorbent, as fresh mobile phase was added to the column in the initial stages of development. The ethanol (50%) extract of sample *Daucus carota* extract was placed over the filter paper disk and used to isolate the active constituents. The crude ethanol extract of sample was subjected to column chromatography over silica gel 100-200 mesh. The column was eluted with solvents of increasing order of polarity. The fractions were collected in 25ml each and allowed to evaporate to get the residue.

Purification of bioactive compound by Thin Layer Chromatography (Kirchner, 1978)

The stationary phase was prepared as slurry with water or buffer at 1: 2 and applied to a glass plate or an inert

plastic or aluminum sheet, as thin uniform layer by means of a spreader such as glass rod or pipette or using a TLC applicator. (0.25 mm thickness for analytical separations and 2 – 5 mm thickness for preparative separations are prepared).

Calcium Sulphate $CaSo_4$.1/2 H_2O; (Gypsum) (10 – 15 %) was incorporated to adsorbent as a binder, as it facilitates the adhesion of the adsorbent to the plate. After application of the adsorbent, the plates were air – dried for 10–15 min and then oven-dried for 10–15 min at 100°C–110°C. The plates were stored in desiccators. The samples were spotted using capillary tubes at 1.5 cm distances between them for preparative TLC, the sample was applied as a band across the layer rather than as a spot. The chromatographic tank was filled with developing solvent to depth of ~1.5 cm and equilibrated for about 5h. The thin layer plate was placed gently in the tank and allowed to stand for about 60 min. making sure the spots did not touch the solvent directly capillary action caused the solvent to ascend as in paper chromatography and the separation of compounds takes place. As the solvent front reached about 1-2 cm from the top of the plate, the plate was removed, solvent front was marked with a pencil immediately and allowed to air dry placing the plate upside down.

Terpenoids

The terpenoids were separated using chloroform and methanol solvent mixture in the ratio of 65:5. The color and Rf values of recorded under visible light.

Results and discussion

The present study of phytochemical investigation revealed that the presence of medicinally active constituents in the extract of *Daucus carota*. The phytochemical characters of the *Daucus carota* was investigated to analyze the preliminary phytochemical constituents qualitatively by the plant extract undergo various chemical test and phytochemical constituents quantitatively analyze through TLC method.

The preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, carbohydrate, phenol, flavonoids, coumarin, chlorogenic acid and terpenoid in ethanolic extract, where as the steroids, tannin, anthocyanin, flavones and saponin are absent in ethanolic extract of *Daucus carota* the result are tabulated in Table 2. The isolated compound, terpenoid showed 0.92 R*f* value in TLC.

Table 2: Qualitative phytochemical analysis of *Daucus carota* extracts.

S. No.	Name of the Test	Phytochemical constituents	Carrot
1	Mayer's test Dragondraff test Wagner Test	Alkaloids	+ + +
2	Molish Test	Carbohydrates	+
3	H_2SO_4	Saponins	-
4	Lead Acetate	Tannins	-
5	Liebermann's	Steroidal Glycosides	-
6	Ammonia	Flavonoids	+
7	Phenol reagent	Phenols	+
8	Chloroform and H_2So_4	Terpenoid	+
9	10% Nacl	Coumarin	+
10	NaOH	Anthocyanin	-

The IR result shows the structure of reduced compound from plant under *Invitro* method using different medium. All the findings are subjected to identify by UV and FT-IR which is a carbon-carbon group as the active component in fractions. The fractions showed in highest peak 1639.27 absorption in the IR spectra and the lowest peak 1102.84 indicated that the compounds have carbon (C=C) and carbon bonds characteristic of terpenoid. This study therefore confirms of Antioxidant compound as an active component in our present investigation (Figs. 1 and 2). The bioactive terpenoids and flavonoids from a similar species, *Daucus littoralis* Smith subsp. *hyrcanicus* Rech.f, an endemic species of Iran, has been reported by Yousefbeyk et al. (2014). Their study isolated four terpenoids, β-sitosterol, stigmasterol, caryophyllene oxide, β-amyrin and also three flavonoids

namely quercetin 3-O-β-glucoside, quercetin 3-O-β-galactoside and luteolin. Recently, Yahyaa et al. (2015) identified and characterized that the terpene synthases are potentially involved in the formation of volatile terpenes in *Daucus carota* roots.

The preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, carbohydrate, phenol, flavonoids, coumarin and chlorogenic acid. This study confirms that terpenoid is an active component in the present investigation.

Fig. 1: UV – Spectral analysis of *Daucus carota* extracts.

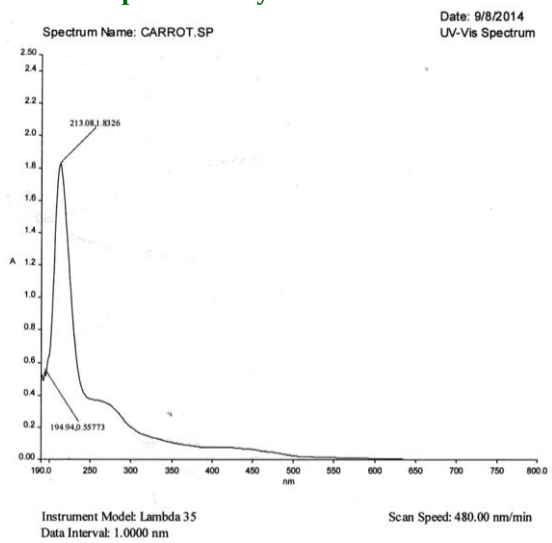
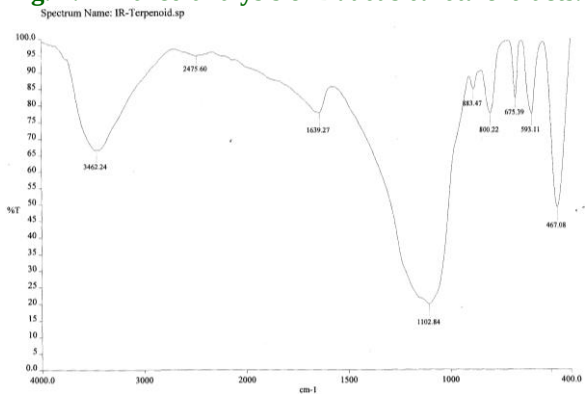


Fig. 2: Infrared analysis of *Daucus carota* extracts.



References

Fraenkel, G.S., 1959. The raison d'Etre of secondary plant substances. Sci. 129, 1466-1470.

Garrod, B., Lewis, B.G., Coxon, D.T., 1978. Cis-heptadeca-1,9-diene-4,6-diyne-3,8-diol, an antifungal polyacetylene from carrot root tissue. Physiol. Plant Pathol. 13(2), 241–246.

Gbile, Z.O., 1986. Ethnobotany, Taxonomy and Conservation of Medicinal Plants. Pp. 13 -29. In: The State of Medicinal Plant Research in Nigeria (Ed.: Sofowora, A.). University of Ibadan Press, Ibadan, Nigeria.

Ghana Herbal Pharmacopeia (GHP), 2007. Council for Scientific and Industrial Research (CSIR). Accra, 295pp.

Harborne, J.B., 1973. Phytochemical methods of analysis. Chapman and Hall, London. 33p.

Kirchner, J.G., 1978. Thin-layer Chromatography. 2nd Edn. Wiley-Interscience, New York.

Rangari, V.D., 2008. Pharmacognosy and Phytochemistry. Second Edn. Career Publications, India. pp.95–97.

Stamp, N., 2003. Out of the quagmire of plant defense hypotheses. Quart. Rev. Biol. 78(1), 23–55.

Wikipedia, 2015. https://en.wikipedia.org/wiki/Carrot.

Yahyaa, M., Tholl, D., Cormier, G., Jensen, R., Simon, P.W., Ibdah, M., 2015. Identification and characterization of terpene synthases potentially involved in the formation of volatile terpenes in carrot (*Daucus carota* L.) roots. J. Agric. Food Chem. 63(19), 4870–4878.

Yousefbeyk, F., Gohari, A.R., Hashemighahderijani, Z., Ostad, S.N., Salehi Sourmaghi, M.H., Amini, M., Golfakhrabadi, F., Jamalifar, H., Amin, G., 2014. Bioactive terpenoids and flavonoids from *Daucus littoralis* Smith subsp. *hyrcanicus* Rech.f, an endemic species of Iran. Daru. 22(1), 12.