RESEARCH PAPER

Accepted 30 December 2005
Revised 09 May 2005
Received 19 October 2004
Indian J. Pharm. Sci., 2005, 67(6): 716-720

Isolation and Hypotensive activity of Three New Phytoconstituents from Seeds of *Daucus carota.*

A. A. SIDDIQUI,* S. M. WANI, R. RAJESH AND V. ALAGARSAMY[1]
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard University, New Delhi-110 062
[1]Department of Pharmaceutical Chemistry, J. S. S. College of Pharmacy, Mysore- 570 015

Three extracts of *Daucus carota* have been prepared and studied for their hypotensive activity. Hydroalcoholic extract was found to exhibit prominent activity when compared to the reference standard minoxidil. In an attempt to isolate the active constituents responsible for this activity, three new phytoconstituents were isolated and their structures were elucidated from spectral evidence (IR, NMR and Mass spectra). Hypotensive activity of these compounds was also studied.

Major efforts have been directed during the last three decades to diagnose and treat patients with hypertension. This certainly contributes significantly to the decreased incidence in cardiovascular diseases observed in developed countries, but much remains to be done, since high blood pressure still represents one of the leading causes of morbidity and mortality world wide[1]. Lowering blood pressure in hypertensive patients requires not only a broad choice of effective and well tolerated medications, but also skills to motivate them to comply with the life-long treatment[2]. In recent years many synthetic drugs are in clinical practice to treat diverse cardiac problems. Among these, hypertension is the major one. Along with the specific action of the drugs on heart, they produce different side effects depending on their mechanism of action sometimes these side effects lead to severe complications. An attempt has now made to screen a traditional herbal medicine to treat this fatal disease with less side effects and the isolation of the phytoconstituents.

Daucus carota (Apiaceae), commonly known as carrot, is a biennial plant, 1-1.5 m height or sometimes more branching from base. It is considered to be native of the sea coasts of S. Europe, but is reported to be of very ancient cultivation. It is cultivated through out the world[3]. According to unani literature, the wild carrot is laxative[4]; seeds are considered to be nervine tonic[4]. In Punjab, the seeds are considered to exhibit aphrodisiac action and given in uterine pain[5]. Carrot has been reported to possess diuretic, nitrogen balance property; it also helps in elimination of uric acid[6]. It is a popular remedy for jaundice in some parts of Europe and their fresh root and seeds are used as aphrodisiac and nervine tonic as well as poultice for foul ulcer, burns and scalds[7]. The oil of carrot seeds has shown antimicrobial property[8,9]. The oil obtained from seeds of carrot exhibited hypotensive activity[10]. The effect of carrot on hormone regulated uterine changes has also been studied[11,12]. Seed oil has been reported to contain bisaboene, cis and trans asarene, asarone aldehyde, rugenol-2-hydroxy-4-methoxy acetophenone and vanillin[13,14].

Inspite of its use in cardiovascular ailments, the hypotensive activity of the seeds of *Daucus carota* is not been explored. In view of this, in the present study various extracts of seeds of *Daucus carota* have been prepared and studied their hypotensive activity. In an effort to isolate the active constituent(s) responsible for this activity, three new phytoconstituents were isolated from hydroalcoholic extract which are not reported in literature[15-19]. Structures of these constituents were characterized by spectral data (IR, NMR and Mass spectra) and evaluated them for hypotensive activity.

*For correspondence
E-Mail: anees_56@yahoo.co.in

MATERIALS AND METHODS

Plant material:

The seeds of *Daucus carota* were procured from Rajdhani seeds corporation, New Delhi. It was authenticated in the department of botany, Jamia Hamdard, New Delhi, India. A voucher specimen has been kept in our laboratory for future reference. The seeds were standardized and powdered.

Melting points were recorded on a Perfit apparatus and are uncorrected. Infrared spectra were recorded on a Jasco-410 Spectrometer by using potassium bromide pellet and nujol mull for solid and semisolid compounds, respectively. NMR spectra were recorded on a Jeol JNM FX-100 FT NMR Spectrometer in duteriated chloroform solvent using tetramethylsilane as internal standard. The FAB mass spectra were scanned on a Jeol SX-102/DA-6000 Mass Spectrometer. m-Nitrobenzyl alcohol (NBA) was used as the matrix for recording mass spectra. The m/z values of only intense peaks have been mentioned.

Preparation of the extract:

The powdered seeds (1 kg) were subjected for Soxhlet hot extraction with petroleum ether (60-80°), the solvent was removed under vacuum and a crude solid mass was obtained. The flowers extracted with petroleum ether were then re-extracted with chloroform and the solvent was removed under vacuum and a crude solid mass was obtained. The same flowers after extraction with chloroform, successively extracted with ethanol-water (7:3) mixture to get solid crude mass. This dried crude extracts (pet ether, $CHCl_3$, EtOH-H_2O (7:3)) were stored in a desiccator. The chemical constituents of the extract were identified by preliminary qualitative analysis and confirmed by thin layer chromatography (TLC) for the presence of steroids, flavonoids, tannins and reducing sugars.

Column chromatography of hydroalcoholic extract:

Hydroalcoholic extract obtained from the seeds was adsorbed on silica gel (60-120 mesh) for column chromatography. The slurry was completely dried to remove any adsorbed moisture on surface. It was then loaded on the top of the column of silica gel packed with petroleum ether. The polarity of the column solvent was gradually increased, starting from petroleum ether to 5% ethanol in chloroform, with the disappearance/appearance of the existing/new spot when visualized on TLC. Various compounds were isolated from extract as given below.

n-triacontane (DC-1):

Elution of the column with benzene: chloroform (1:1) furnished yellow semisolid compound DC-1 (55 mg) it was recrystallised from methanol. R_f 0.6 (benzene:chloroform, 1:3); IR (KBr, cm^{-1}): 2954 and 2853 (CH); NMR ($CDCl_3$,δ ppm): 1.45 (brs, 2H, CH2), 1.21 (brs, 54H, 27CH2), 0.93 (t, 3H, J=6.1 Hz, Me-1), 0.87 (t, 3H, J=6.0 Hz, Me-30); +ve ion FAB-MS (m/e, Relative intensity): 422 $[M]^+$ ($C_{30}H_{62}$) (57.2).

n-hentriacontane (DC-2):

Elution of the column with chloroform:methanol (49:1) yielded yellow powder of compound DC-2 (70 mg). R_f 0.4 (benzene:methanol, 9:1); mp 40-42°; IR (KBr, cm^{-1}): 2954, 2854 (CH); NMR ($CDCl_3$, δ ppm): 1.41 (brs, 4H, 2 CH_2), 1.21 (brs, 54H, 27CH_2), 0.87 (t, 3H, J=6.0 Hz, Me-1), 0.83 (t, 3H, J=6.1 Hz, Me-31); +ve ion FAB-MS (m/e, Relative intensity): 436 $[M]^+$ ($C_{31}H_{64}$) (12.7).

β-sitosterol-3-O-β-D-glucoside (DC-3):

Elution of the column with benzene:chloroform (1:1) furnished colourless crystals of DC-3. It was recrystallised from chloroform:methanol (3:1) mixture; R_f 0.75 (methanol); mp 150°; IR (KBr, cm-1): 3450 (OH), 1620 and 1030 (C=C); NMR ($CDCl_3$, δ ppm): Table 1; +ve ion FAB-MS (m/e, Relative intensity): 414 $[M]^+$ ($C_{29}H_{50}O$), 396 $[M-H_2O]^+$ (11), 381 [396-CH_3 (7)], 367 [381-CH_3]$^+$ (3.6), 329 [M-side chain, fission via 23 (24)]$^+$ (5), 315 [M-side chain, fission via 22 (23)]$^+$ (5), 301 [M-side chain, fission via 17 (20)]$^+$ (15), 283 [301-H_2O] $^+$ (10), 273 $[M-C_{10}H_{21}]^+$ (15), 163 (30), 159 (10), 145 (10), 128 (20), 107 (15), 99 (10), 85 (10), 83 (15), 69 (50), 67 (50), 57 (90), 44 (100). fig 1.

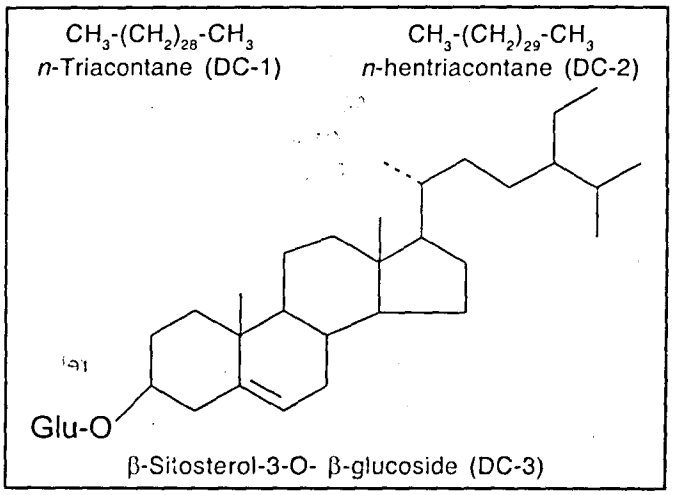

Fig. 1: Chemical Structures of isolated phytoconstituents of *Daucus carota*

TABLE 1: NMR SPECTRAL DATA OF COMPOUND DC-3

Position	NMR
3	3.577 d (1/2 w =16.0) α -H
6	5.36 d (6.0)
18	0.68 s Me
19	1.01 s Me
21	0.94 d (6.8) Me
26	0.821d (96.0) Me
27	0.84 d (6.0) Me
29	0.90 d (6.0) Me
	* 2.428-2.936 m (1H)
	* 2.027-1.762 brm (6H)
	*1.695 1.489 brm (8H)
	* 1.417-1.00 brm (13H)
	Sugar protons
1'	4.443 d (7.5) β-linkage,anomeric proton
2'	3.862 d (6.0)
3'	4.425 d (7.5)
4'	4.40 d (7.5)
5'	3.758 m (1/2 w = 14.25)
6'a	3.482 dd (5.1, 4.8)
6'b	3.30 dd (2.1, 2.4)

*Assignable to methylene and methane protons

Hypotensive activity:

The crude extracts and isolated compounds were evaluated for hypotensive activity. Student-t-test was performed for all the activities to ascertain the significance of the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% sodium CMC as vehicle) in the same route of administration. Each group consisted of five animals. The animals were maintained in a colony cages at 25±2°, relative humidity of 45-55%, maintained under 12 h light and dark cycle and were fed with standard animal feed. All the animals were acclimatized for a week before use.

The Institutional Animal Ethics Committee of Jamia Hamdard, Hamdard University, New Delhi has approved the experimental protocol (173/CPCSEA, P. NO. 48). Petroleum ether, chloroform and ethanol-water (7:3) extracts of the seeds of *D. carota* were tested for hypotensive activity in normotensive rats, non-invasively, by tail-cuff method using LE 5001 Pressure Meter[20]. Adult Wistar rats of either sex, weighing 200-250 g, were used for the screening of extracts for hypotensive activity. Suspension of all the three extracts was prepared by using 1% sodium CMC and was administered orally at dose levels 50, 100 and 200 mg/kg animal body weight to different groups of rats, each containing five. Control group received an equivalent quantity of sodium CMC suspension.

After 3 h of administration of the extract, animal was shifted to the restrainer, which restricts the movement of animal. The tail was cleaned with moist cotton to remove the dirty matter and talcum powder was sprayed on tail to make its surface smooth. A tail-cuff and pulse transducer was fixed around the tail. Initially animal shows particular pulse level. When this pulse rate was within the normal range, start switch was pressed and the recorder records the blood pressure as SBP (systolic blood pressure), DBP (diastolic blood pressure), and MABP (mean arterial blood pressure), the same displayed on the monitor. The Blood pressure was read from the precaliberated monitor. Percent reduction in blood pressure was calculated in comparison to minoxidil, which was used as standard, at the same dose levels. Results were analyzed by using student's t-test. Percent reduction in blood pressure after administration of the extracts is shown in Table 2.

The same procedure has been adopted for determining reduction in blood pressure for isolated constituents and the results are depicted in Table 3.

RESULTS AND DISCUSSION

The seeds of *D. carota* were collected, processed and extracted with various solvents and the crude extracts were subjected to the hypotensive by tail-cuff method using wister rats. The results of hypotensive activity studies of crude extracts (Table 2) indicate that the hydroalcoholic extracts exhibited prominent activity when compared to the standard minoxidil, hence hydroalcoholic extract was column chromatographed with the aim to isolate pure constituents responsible for hypotensive activity. Following compounds were isolated and structurally characterized on the basis of spectral data.

Elution of column with chloroform-methanol (49:1) gave compound DC-1. The FAB mass spectrometry suggested

TABLE 2: HYPOTENSIVE ACTIVITY OF VARIOUS EXTRACTS OF SEEDS OF *DAUCUS CAROT*

Extract	50 mg/kg body weight			100 mg/kg body weight			200 mg/kg body weight		
	SBP	DBP	MABP	SBP	DBP	MABP	SBP	DBP	MABP
Petroleum ether	7.6	11.7	10.3	11.0	12.7	12.1	13.3	13.1	13.1
	±0.9	±0.9	±0.9	±0.8	±0.9	±0.9	±0.5	±0.8	±0.6
Chloroform	2.5	1.9	2.1	9.0	7.6	8.2	13.1	11.5	11.8
	±0.8	±0.5	±0.6	±0.7	±0.8	±0.8	±0.9	±0.7	±0.9
Ethanol-water (7:3)	6.3	8.1	7.4	8.6	12.2	11.1	22.8	17.3	19.4
	±0.1	±1.2	±1.1	±0.9	±1.1	±1.2	±0.5	±0.8	±0.6
Minoxidil	6.35	8.24	6.60	46.0	32.0	43.3	58.7	57.7	58.4
	±0.8	±1.0	±1.0	±0.8	±1.4	±1.5	±1.2	±0.6	±0.3

Each value represents the mean±SEM (n=5). Significance levels P<0.01. SBP Systolic blood pressure, DBP- Diastolic blood pressure, MABP Mean arterial blood pressure

the molecular formula $C_{30}H_{62}$ (m/z 422, $[M]^+$). Its IR spectrum did not show any absorption band in the functional group region, suggesting hydrocarbon nature of the compound. NMR spectrum of DC-1 showed two three-proton triplets at δ 0.93 (J=6.1 Hz) and 0.87 (J=6.0 Hz), assigned to primary methyl protons at C-1 and C-31 position, respectively. Two multiplets at δ 1.21 and 1.45 were accounted to twenty-seven and one methylene groups, respectively. The mass spectrum of DC-1 showed molecular ion peak at m/z 422 $[M]^+$ (57.2) in accordance with acyclic hydrocarbon possessing molecular formula $C_{30}H_{62}$. On the basis of above evidences, the compound DC-1 was assigned the structure as *n*-triacontane.

Elution of column with chloroform-methanol (49:1) furnished colorless amorphous compound DC-2. The FAB mass spectrometry suggested the molecular formula $C_{31}H_{64}$ (m/z 436, $[M]^+$). Its IR spectrum did not show any absorption band in the functional group region, suggesting the hydrocarbon nature of the compound. The NMR spectrum of DC-2 showed to two three-proton triplets at δ0.83 (J=6.1 Hz) and 0.87 (J=6.0 Hz), assigned to primary methyl group at C-1 and C-31 position, respectively. Two multiplets at δ 1.21 and 1.41 were associated with twenty-seven and two methylene groups, respectively. The mass spectrum of DC-2 showed molecular ion peak at m/z 436 $[M]^+$ (12.7) in accordance with the acyclic hydrocarbon possessing molecular formula $C_{31}H_{64}$. On the basis of above evidences, the compound DC-2 was identified as *n*-hentriacontane.

The compound DC-3 was obtained as colorless crystals after eluting the column with benzene:chloroform (1:1). It gave positive Leibermann-Burchard test, and Molisch's test suggesting the presence of steroidal glycoside. The IR

TABLE 3: HYPOTENSIVE ACTIVITY OF ISOLATED CONSTITUENTS OF SEEDS OF *DAUCUS CAROTA*

Constituents	25 mg/kg body weight			50 mg/kg body weight		
	SBP	DBP	MABP	SBP	DBP	MABP
DC-1	1.9±0.3	3.9±1.2	3.4±0.2	3.7±0.9	4.3±0.9	4.2±0.9
DC-2	2.5±1.2	3.9±1.1	3.3±1.1	3.7±0.5	6.2±0.7	5.1±0.7
DC-3	6.8±1.4	4.3±0.9	5.6±0.9	5.49 ±0.4	3.5±0.8	4.3±1.2
Minoxidil	14.1±0.7	14.5±1.1	14.7±0.9	29.4±0.8	32.4±1.1	31.4±0.9

Each value represents the mean±SEM (n=5). Significance levels P<0.01. SBP Systolic blood pressure, DBP- Diastolic blood pressure, MABP Mean arterial blood pressure, StandardMinoxidil

spectrum of the compound DC-3 exhibited absorption bands at 3450, 1030 and 1620 cm^{-1} indicating the presence of alcoholic hydroxyl group and double bond in the compound. The presence of double bond was further confirmed by its NMR spectrum which showed a doublet at δ 5.36 (J=6.0 Hz). The NMR spectrum also showed the presence of six signals of three protons each at δ 0.68 (C-18), 1.01 (C-19), 0.94 (C-21), 0.82 (C-26), 0.84 (C-27), 0.9 (C-29). The NMR spectrum showed a broad multiplet at δ 3.57 due to carbinolic proton at C-3 position. Its half width of multiplet was found to be 16.0 Hz indicating the -orientation of the carbinolic proton. The presence of hydroxyl group was assigned C-3 position, biogenetically and on the basis of mass spectrum, which was found to, linked with sugar moiety. The presence of double bond was found at $\Delta^{5(6)}$ on the basis of mass fragmentation pattern of the aglycone which exhibited sharp peak at m/z 69 and 85. The hydrolysis of the compound with 10% hydrochloric acid afforded an aglycone with the help of co-paper chromatography. The sugar was found to be linked with the aglycone moiety through β-linkage as evidenced by a doublet at δ 4.4 (J=7.5 Hz) of the anomeric proton in the NMR spectrum. The other signals of the sugar were found complexed with glucose proton in the NMR spectrum.

The aglycone obtained on hydrolysis showed molecular ion peak at m/z 414 corresponding to molecular formula $C_{29}H_{50}O$. The mass spectrum exhibited the characteristic peak at m/z 396 ($M-H_2O$), 329 ($M-C_6H_{11}$), 301($M-C_8H_{17}$), and 273 ($M-C_{10}H_{24}$). The mass spectrum also proved the absence of double bond in the side chain of ring A, C and D. The ions at m/z 69 and 85 indicated further the presence of double bond at $\Delta^{5(6)}$. On the basis of above spectral data, compound DC-3 was assigned the structure as β-sitosterol-3-O-β-D-glucoside.

The above isolated constituents, when subjected to the hypotensive activity (Table 3), all the compounds shown mild hypotensive activity. These findings suggested that there must be synergistically acting constitutent(s) present in crude extract, responsible for its hypotensive activity and these compounds are only effective in combination with each other, and not alone. Hence further more pharmacological investigations are required to find out proper combination of two or more constituents, which may shows the expected hypotensive activity.

REFERENCES

1. De, S.M.A., **Drugs**, 1997, 54, 801.
2. Ali, M.S. and Azhar, I., **Hamdard Medicus**, 2000, 2, 72.
3. Bishayee A. and Chatterjee M., **Fitoterapia**, 1993, 64, 261
4. Mhaskar, K.S. and Bletter, E., Eds., In; Kirtikar and Basu's Illustrated Indian Medicinal Plants, Sri Satguru Publications, New Delhi, 1989, 462.
5. Nadkarni, K.M., Eds., In; Indian Materia Medica, Popular Prakashan, Mumbai, 1976, 442.
6. Shastrm, B.N., Eds., In; Wealth of India-Raw Materials, CSIR Publication, New Delhi, 1952, 20.
7. Norton, S.P., Srivastava, N. and Jain, S., **J. Chem.**, 1989, 5, 237.
8 Dhar, V.J., **Fitoterapia.**, 1990, 61, 255.
9. Bhargava, A.K., Chauhan, C.S. and Ali, S. M., **Indian. J. Pharm.**, 1967, 28, 127.
10. Bhatt, C., Solberg, M. and Ceponis, M., **J. Food Sci.**, 1983, 48, 762.
11. Khanna, R.K. and Johri, J.K., **Natl. Acad. Sci. Lett.**, 1991, 14, 142.
12. Pigulevskii, G.V. and Motskus, K.V., **Zh. Priklad. Khim.**, 1962, 35, 1355.
13. Archive, M.C., **Perfum. Flavor**, 1992, 17, 1.
14. Dwived, S., **Flovour Frag.** J., 1991, 6, 295.
15. Derimov, S., **Zh. Priklad. Khim.**, 1963, 36, 741.
16. Chaterjee, A. and Chaudhary, B., **J. Chem. Soc.**, 1961, 4, 2246.
17. Rastogi, R.P., Mehrotra, B.N., Eds., In; Compendium of Indian Medicinal Plants Central Drug Research Institute, Luknow Publication, 1970, 9, 259.
18. Keys, A.J. and Leaver, C., **Nature**, 1971, 232, 423.
19. Mohammad, A. and Javed, A., **Pak. J. Sci. Ind. Res.**, 1977, 20, 103.
20. Rodrigues, B., Goyal, R.K. and McNeill, J.H., **J. Pharm. Exp. Ther.**, 1986, 237, 292.