NPC Natural Product Communications

2013
Vol. 8
No. 8
1123 - 1126

Absolute Configuration of Falcarinol (9*Z*-heptadeca-1,9-diene-4,6-diyn-3-ol) from *Pastinaca sativa*

Mireia Corell[a], Emile Sheehy[b], Paul Evans[b], Nigel Brunton[c] and Juan Valverde[*,c]

[a]*Department of Agroforestry Sciences, School of Technical Agricultural Engineering, University of Seville, Ctra de Utrera, C.P. 41013, Spain*

[b]*Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, D4, Ireland*

[c]*Teagasc Food Research Centre, Ashtown, D15, Dublin, Ireland*

juan_valverde@about.me

Received: October 11th, 2012; Accepted: February 18th, 2013

Falcarinol (9*Z*-heptadeca-1,9-diene-4,6-diyn-3-ol; (**1**) is a polyacetylene commonly found in several plant families. The absolute configuration of naturally occurring **1** is not clear and contradictory results have been reported in the literature. Determination of the absolute configuration of **1** from *Pastinaca sativa* L. was carried out. Isolation of 95% pure **1** was performed via successive fractionation and preparative-HPLC. A racemic mixture comprised of 3*R*-**1** and 3*S*-**1** was synthesized in order to confirm the absolute configuration of the isolated natural product using chiral HPLC. Based on a combination of chiral HPLC and specific rotation, **1** present in *P. sativa* was found to have a 3*R* absolute configuration (i.e. (3*R*, 9*Z*)-heptadeca-1,9-diene-4,6-diyn-3-ol).

Keywords: Falcarinol (9*Z*-heptadeca-1,9-diene-4,6-diyn-3-ol), Absolute configuration, *Pastinaca sativa*, Chiral chromatography.

Falcarinol (**1**) is a bis-acetylene that is part of a wider family of compounds known as polyacetylenes, some of which contain more than one alkyne. Polyacetylenes are commonly found in several plant families [1], including commonly consumed members of the *Apiaceae* family such as *Daucus carota*, *Pastinaca sativa* and *Petroselinum crispum*. Falcarinol (**1**) and other polyacetylenes have been the subject of several studies due to their interesting biological properties. In plants, polyacetylenes play an important role in the prevention of fungal root infections. When consumed by humans, polyacetylenes have documented health benefits, which include anti-inflammatory [3], anti-platelet-aggregatory [4], antifungal [5], antiviral [6] and anticancer effects [7]. Recently, polyacetylenes were associated with a reduction in the risk of developing certain types of cancer and other important diseases [8].

Falcarinol (**1**) was first isolated from *Panax ginseng* roots by Takahashi [9, 10] and was named panaxynol. Later Bohlmann and co-workers isolated a compound with a similar structure to panaxynol from *Falcaria vulgaris* [11]. At the same time, Crosby isolated a natural toxicant from *Daucus carota* with a very similar structure and named it carotatoxin [12]. The three compounds have the same chemical structure, but the use of different trivial names has, historically, led to confusion. This lack of clarity has been compounded by the fact that both enantiomers (3*R* and 3*S*) have been isolated from different plants (Figure 1). In addition, the stereochemical determination of these types of polyacetylenes is problematic when current analytical techniques such as the Mosher´s ester and circular dichroism methods are used. One reason for this is that these techniques are less reliable when secondary alcohols are flanked by two unsaturated chromophores [13,14].

It is still common to find inaccurate chemical structures in recent literature. The absolute stereochemistry of (+) and (–)-falcarinol was finally confirmed by stereodefined synthesis [14-17] and similar arguments have confused workers with falcarindiol [18-20].

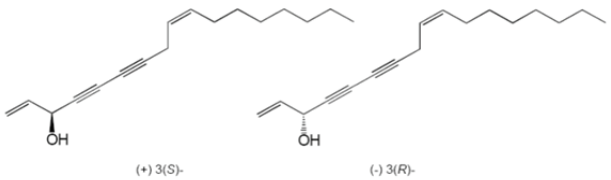


Figure 1: Structure of two possible stereoisomers of 9*Z*-heptadeca-1,9-diene-4,6-diyn-3-ol.

Recent publications have given contradictory assignments to this class of natural products. For example, Yadav and co-workers have chemically synthesized oploxynes A, B and their C-10 epimers [21], and this cast doubts on the previously assigned stereochemistry of oploxyne B, which was isolated from the stems of *Oplopanax elatus* [22].

3*R* derivatives of **1** were described previously for isolates generated from *Apiaceae* and *Asteraceae* genera. However, the 3*S* configuration for **1** was related to isolates generated from *Araliaceae* [11, 20]. Knowledge of the absolute configuration of falcarinol (**1**) is necessary. Indeed, a recent report indicated that significant differences in biological properties were found with stereoisomeric polyacetylenic structures [23].

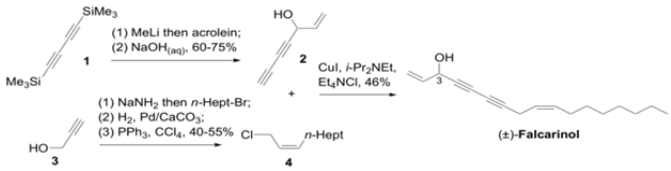
Therefore, the aim of this work was to determine the absolute configuration of **1** from *Pastinaca sativa* and confirm the initial assumptions that the 3*R* enantiomer is present in plants of the *Apiaceae* family. A newly developed chiral HPLC method was employed to achieve this aim. This method was used for the separation of a mixture of chemically synthesized enantiomers and for analyses of the isolated natural product and a "spiked-mixture" of the synthetic racemic mixture with the natural product. In this way the structure of the natural compound was assigned based on retention time rather than the Mosher's ester technique. As falcarinol is not commercially available, a new preparative HPLC

method was used to isolate falcarinol (**1**) of approximately 95% purity from roots of *P. sativa*.

A HPLC chromatogram of an ethyl acetate extract from freeze-dried powder pre-purified using flash chromatography showed a distinct peak with a retention time of 17.25 min, detected at a wavelength of 205 nm. The absorbance spectrum between 190 and 320 nm of this peak showed the typical profile of **1**; one large and intense band with a maximum at 205 nm, and three smaller bands with maxima at 230, 245 and 260 nm, respectively [24]. A number of other falcarinol isolation techniques have been reported in the literature [24-26]. However, some of these, including column, thin layer and multilayer coil counter current chromatography were reported as either time consuming or requiring special equipment, and are, therefore, less useful for large scale isolation than preparative HPLC. The addition of a pre-purification step using normal phase flash chromatography meant that a relatively short run time (33 min) could be used, as many of the impurities (mainly carotenes) were removed from the crude extract. HPLC analysis of this fraction yielded a single peak that corresponded to **1** and constituted >95% of the total detectable area by HPLC and by ^{1}H NMR spectroscopy.

The prep-HPLC procedure used in the present study had a relatively short run time in comparison with other reported methods, was easy to implement and was able to produce relatively large quantities of material. The ability to isolate relatively large quantities of **1** from a plant source is essential in order to carry out biological studies *in vivo* as **1** is not commercially available. The total yield of falcarinol was slightly higher than 2 mg per kg of root of *P. sativa*. This is similar to that reported by Crosby for *Daucus carota* [12]. Whilst the isolation method presented here was effective, the absolute yield is a reflection of the low abundance of the compound in the natural product. Therefore, alternative synthetic routes for this compound are necessary. However, the determination of the absolute configuration of the natural product is essential before developing strategies to synthesize **1** as the mixture of enantiomers could have different biological properties than those of the isolated enantiomers.

Using the synthetic route outlined in Scheme 1, a racemic mixture of **1** was synthesized. The individual stereoisomers comprising racemic **1** proved separable using an OJ-H chiral analytical column. Since previous studies [16] had uncovered that the (–)-isomer had a shorter retention time than the corresponding (+)-isomer, this enabled investigation of the absolute stereochemistry presented by **1** from this natural source. Based on the stereodefined syntheses reported by Cai and co-workers [14], it is now well-established that the (+)-enantiomer of falcarinol possesses a 3*S*-stereogenic centre.



Scheme 1

Figure 2 shows the chiral HPLC analyses of three samples, the synthetic mixture of (*R*)- and (*S*)- falcarinol (Figure 2, graph A) showing complete separation of both enantiomers with retention times of 6.8 and 8.0 min, with peak areas of 44% and 56%, respectively. The isolated **1** from the root of *P. sativa* (Figure 2, graph B) shows that the natural product was comprised of only one

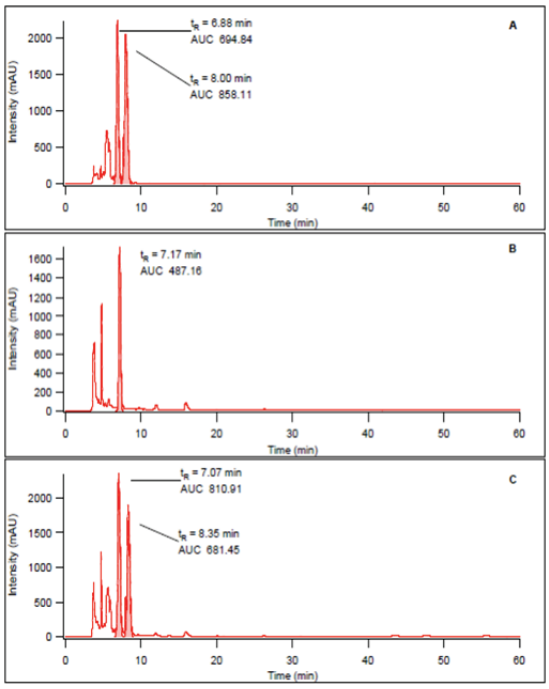


Figure 2: Chiral HPLC analyses on OJ-H column, eluted isocratically with *n*-heptane-EtOH, 95:5, at a flow rate of 1.0 mL/min. The elution was monitored at 210 nm. Graph A: Synthetic mixture of 3*R* and 3*S;* Graph B: falcarinol isolated from *Patinaca sativa* roots; Graph C: Synthetic mixture of 3*R* and 3*S* spiked with the isolated falcarinol from *P. sativa* roots.

enantiomer, with a retention time of 7.1 min and some impurities. The retention time for the isolated natural product was not sufficient to determine its absolute configuration and a "spiked" synthetic mixture with the natural **1** was carried out. Lastly, purification of the synthetic mixture, spiked with the natural product was performed (Figure 2, graph C) and a significant increase in the area under the curve of the first peak was observed.

The assignment of the eluting peaks in chiral chromatography was supported by the optical rotation values recorded for the isolated falcarinol, the racemic mixture, and the spiked racemic mixture. Isolated falcarinol optical rotation showed negative (laevorotatory) properties ($[\alpha]_D^{20}$ – 37, *c* = 1.0, CH_3Cl), which is unambiguously [14] correlated to the *R* absolute configuration at C-3 [16]. On the other hand, the optical rotation for the synthetic racemic mixture showed slightly positive (dextrorotatory) properties and corresponded to an 18% enantiomeric excess of (*S*)-falcarinol over (*R*)-falcarinol. This was in agreement with the value of the ratio between the areas under the curve of both peaks obtained by chiral chromatography (*R* / *S* = 0.8). The enantiomeric mixture spiked with the isolated natural falcarinol again showed negative properties (*ca* 20% enantiomeric excess of (*S*)-falcarinol over (*R*)-falcarinol) that once more correlated well with the *R/S* ratio of the areas under the curve obtained by chiral chromatography (*R* / *S* = 1.19). This indicates that the absolute configuration of falcarinol (**1**) from the root of *P. sativa* corresponds to the (*R*)- enantiomer.

In summary, the absolute configuration of natural falcarinol (**1**) from *P. sativa* root was unambiguously determined to be 3(*R*)-using a combination of chiral HPLC and polarimetry. Inaccurate chemical assignments for **1** and its related compounds still occur in the literature and we believe these results help to reduce the level of confusion concerning the structure of this natural product. The absolute configuration of **1** is necessary in order to understand the biochemical mechanism of action because different stereisomeric

polyacetylenic structures can lead to different biological properties. The methods described herein could be easily applied for determining relatively rapidly and unequivocally the absolute configuration of **1** from other sources. Low abundance of **1** in natural sources highlights the importance of developing alternative and more cost effective synthetic routes. Knowledge on the absolute configuration of a natural product and its specific biological properties will impact on the strategies of how they are chemically synthesized.

Experimental

Solvents and reagents: All reagents used for the chemical synthesis of (±)-heptadeca-1,9-*Z* diene-4,6-diyn-3-ol [(±)-**1**] were purchased from Sigma Aldrich. Solvents for the extraction, isolation and chromatographic separation of the natural product were HPLC grade from Romil (UK).

Isolation of falcarinol: Frozen, non-peeled roots were sliced vertically into *ca* 5 mm disks using a slicer (Berkel 800, UK). Once samples were sliced they were immediately lyophilized using a freeze-drier Model A6/14 (Frozen in Time Ltd, UK). Freeze-dried sliced roots were milled into a fine powder using a blender (Robocoupe Blixer 3000, France). The milled powder was then used for extraction with ethyl acetate overnight with constant shaking (5 L of ethyl acetate were used per kg of freeze-dried powder). The extract was filtered and the residue re-extracted with the same proportion of ethyl acetate. The filtered extracts were dried in a large scale rotary evaporator R-220 (Büchi, Switzerland) obtaining a crude oil that represented *ca* 2% of initial mass.

The root extract was pre-purified using flash chromatography (IntelliFlash 310 Analogix, Varian, USA). The column used for the pre-purification was a Superflash pre-packed silica column (SF65-200 g, 50 µm, Varian, USA). The following fractions were collected at a flow rate of 100 mL/min (F0) 100% cyclohexane until a dark-red, brown band was completely removed; (F1) elute with 5% ethyl acetate in cyclohexane for 15 min; (F2) elute with 10% ethyl acetate in cyclohexane for 15 min; (F3) elute with 20% ethyl acetate in cyclohexane for 30 min, and (F4) elute with 100% ethyl-acetate. All fractions were monitored in a HPLC (Agilent 1100 series) at a wavelength of 205 nm using a method recently reported [27]. The 5% ethyl acetate in cyclohexane fraction (F1) contained **1**.

Isolation of **1** from F1 was performed using a Prostar 218 Prep-HPLC system (Varian, USA) equipped with a diode array detector. Preparative HPLC separation was carried out on a Luna 5µ C-18(2) (100 x 21.20 mm, 100 Å, 5 µm) Prep HPLC column (Phenomenex, USA). Elution was performed at a constant flow rate of 10 mL/min. The solvent system consisted of 70% acetonitrile in water for 5 min, changed linearly over 13 min to 86% acetonitrile, finally to 95% acetonitrile for 2 min; this was maintained isocratically for 8 min. The percentage of acetonitrile was changed to 70% over 2 min. Falcarinol was detected by absorbance at 205 nm. Falcarinol was collected between 16 and 20 min. The purity of the collected peak was analyzed with a PDA detector coupled to the Prep-HPLC and through ^{1}H NMR spectroscopy.

Synthesis of (±)-heptadeca-1,9Z diene-4,6-diyn-3-ol: Following our recently reported protocol [16], a racemic mixture of falcarinol was prepared in a five-pot seven-step synthetic sequence (Scheme 1). Briefly, the non-volatile butadiyne surrogate **1** (in Scheme 1) was lithiated with methyl lithium (1 equiv.) and quenched with the α,β-unsaturated aldehyde, acrolein. Basic work-up in aqueous sodium hydroxide gave alcohol **2** (in Scheme 1). Propargyl alcohol **3** (in Scheme 1) was alkylated with 1-bromoheptane and the now internal alkyne was stereoselectively reduced with molecular hydrogen and Lindlar's catalyst ($Pd/CaCO_3$) to afford the *Z*-allylic alcohol, which was converted into the chloride **4** (in Scheme 1) following an Appel reaction. Coupling under Cu (I) mediated conditions gave racemic falcarinol in moderate, but reproducible yield. The main issue with this alkylation is that a by-product (not shown) resulting from an S_N2' process is also formed.

NMR analysis: ^{1}H NMR spectra were recorded on a BrukerTM Avance Spectrometer (Coventry, UK) at 500.162 MHz and 300 K using a 5 mm PABBO Broad Band Observed probe. The acquired spectra were line broadened (0.5 Hz), and manually phased using TOPSPIN v 1.3 software (Bruker, Coventry, UK) and baseline corrected. The chemical shift of the spectrum was then calibrated from the residual chloroform signal at δ 7.26 ppm. ^{13}C NMR spectra were recorded at 125.3 MHz and 300 K using a 5 mm PABBO Broad Band Observed probe.

Chiral HPLC: Separation of enantiomers was performed using a HPLC (Agilent 1200, USA) equipped with diode array detector and fitted with a polysaccharide coated chiral stationary phase, Chiralcel OJ-H chiral column (250 x 4.6 mm, 5 µm Daicel Chemical Industries, France). Five µL of sample was eluted isocratically with *n*-heptane - EtOH (95:5) during 60 min at a constant flow of 1 mL /min and monitored at 254, 210 and 230 nm.

Heptadeca-1,9*Z*-diene-4,6-diyn-3-ol

$[\alpha]_D^{20}$: – 37 (*c* 1.0, CH_3Cl).
UV/Vis λ_{max} (MeOH) nm (log ε): 205 (10.8), 230 (2.1), 245 (2.0) 260 (1.8).
^{1}H NMR (500 MHz, $CDCl_3$): δ 5.94 (1H, ddd, J = 16.9, 10.2, 5.4 Hz, CH-2), 5.55 – 5.49 (1H, m, CH-10), 5.46 (1H, dt, J = 17.2, 1.1 Hz, CH_a-1), 5.41 – 5.34 (1H, m, CH_2-9), 5.24 (1H, dt, J = 10.1 Hz, CH_b-1), 4.91 (1H, brd, CH-3), 3.03 (2H, d, J = 6.9 Hz, CH_2-8), 2.02 (2H, q, J = 7.2 Hz, CH_2 -11), 1.42 – 1.32 (2H, m, CH_2 -12), 1.32 – 1.20 (8H, m, CH_2 -16-13), 0.88 (3H, t, J = 6.9 Hz, CH_3-17).
^{13}C NMR (126 MHz, $CDCl_3$): δ 136.16, 133.16, 121.85, 117.07, 80.31, 74.21, 71.32, 63.99, 63.59, 38.86, 31.81, 29.17, 27.19, 22.64, 17.68, 14.09.

Supplementary data: ^{1}H and ^{13}C NMR spectra of isolated (-) 3*R*-falcarinol from *Pastinaca sativa* and the ^{1}H and ^{13}C NMR spectra of the synthetic (+)-3*S*, (-)-3*R* mixture are available on line.

Acknowledgments - The Irish Department of Agriculture, Food and Marine (FIRM 06/NITARFC6) and the University of Seville are gratefully acknowledged for financial support of this work.

References

[1] Minto RE, Blacklock BJ. (**2008**) Biosynthesis and function of polyacetylenes and allied natural products. *Progress in Lipid Research*, ***47***, 233-306.
[2] Christensen LP. (**1998**) Biological activities of naturally occurring acetylenes and related compounds from higher plants. *Recent Research Developments in Phytochemistry*, ***2***, 227–257.
[3] Tanaka N, Matsuura E, Terahara N, Ishimaru K. (**1999**) Secondary Metabolites in Transformed Root Cultures of *Campanula glomerata*, *Journal of Plant Physiology*, ***155***, 251-254
[4] Teng C-M, Kuo S-C, Ko F-N, Lee J-C, Lee L-G, Chen S-C, Huang T-F. (**1989**) Antiplatelet actions of panaxynol and ginsenosides isolated from ginseng. *Biochimica et Biophysica Acta (BBA) - General Subjects*, ***990***, 315-320.

[5] Lechner D, Stavri M, Oluwatuyi M, Pereda-Miranda R, Gibbons S. (**2004**) The anti-staphylococcal activity of *Angelica dahurica* (Bai Zhi). *Phytochemistry*, ***65***, 331-335.
[6] Zhang H, Lu Z, Tan GT, Qiu S, Farnsworth NR, Pezzuto JM, Fong HHS. (**2002**) Polyacetylene ginsenoside-Ro, a novel triterpene saponin from *Panax ginseng*, *Tetrahedron Letters*, ***43***, 973-977.
[7] Matsunaga H, Katano M, Yamamoto H, Fujito H, Mori M, Takata K. (**1990**) Cytotoxic activity of polyacetylene compounds in *Panax ginseng* C. A. MEYER, *Chemical & Pharmaceutical Bulletin*, ***38***, 3480-3482
[8] Christensen LP, Jensen M, Kidmose U. (**2006**) Simultaneous determination of ginsenosides and polyacetylenes in American ginseng root (*Panax quinquefolium* L.) by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, ***54***, 8995-9003.
[9] Takahashi M, Isoi M, Yoshitaka K, Yoshikura M. (**1964**) Studies on the components of *Panax ginseng* C. A. Meyer. II. *Yakugaku Zasshi*, ***84***, 752-756.
[10] Takahashi M, Yoshikura M. (**1964**) Studies on the components of *Panax ginseng* C. A. Meyer. III, *Yakugaku Zasshi*, ***84***, 757-759
[11] Bohlmann F, Niedballa U, Rode K-M. (**1966**) Polyacetylenverbindungen. CXVIII. Über neue polyine mit C17-Kette, *Chemische Berichte*, ***99***, 3552-3558.
[12] Crosby DG, Aharonson N. (**1967**) The structure of carotatoxin, a natural toxicant from carrot. *Tetrahedron*, ***23***, 465-472.
[13] Kobayashi M, Mahmud T, Umezome T, Wang W, Murakami N, Kitagawa I. (**1997**) The absolute stereostructures of the polyacetylenic constituents of ginseng Radix Rubra, *Tetrahedron*, ***53***, 15691-15700.
[14] Zheng G, Lu W, Aisa HA, Cai J. (**1999**) Absolute configuration of falcarinol, a potent antitumor agent commonly occurring in plants. *Tetrahedron Letters*, ***40***, 2181-2182.
[15] Mayer SF, Steinreiber A, Orru RVA, Faber K. (**2002**) Chemoenzymatic asymmetric total syntheses of antitumor agents (3*R*,9*R*,10*R*)- and (3*S*,9*R*,10*R*)-panaxytriol and (*R*)- and (*S*)-falcarinol from *Panax ginseng* using an enantioconvergent enzyme-triggered Cascade reaction. *The Journal of Organic Chemistry*, ***67***, 9115-9121.
[16] McLaughlin NP, Butler E, Evans P, Brunton NP, Koidis A, Rai DK. (**2010**) A short synthesis of (+) and (-)-falcarinol. *Tetrahedron*, ***66***, 9681-9687.
[17] Matsuura H, Saxena G, Farmer SW, Hancock REW, Towers GHN. (**1996**) Antibacterial and antifungal polyine compounds from *Glehnia littoralis* ssp. *leiocarpa. Planta Medica*, ***62***, 256-259.
[18] Ratnayake AS, Hemscheidt T. (**2002**) Olefin cross-metathesis as a tool in natural product degradation. The stereochemistry of (+)-falcarindiol, *Organic Letters*, ***4***, 4667-4669
[19] Lemmich E. (**1981**) The absolute configuration of the acetylenic compound falcarindiol. *Phytochemistry*, ***20***, 1419-1420.
[20] Bernart MW, Cardellina JH, Balaschak MS, Alexander MR, Shoemaker RH, Boyd MR. (**1996**) Cytotoxic falcarinol oxylipins from *Dendropanax arboreus*. *Journal of Natural Products*, ***59***, 748-753.
[21] Yadav JS, Boyapelly K, Alugubelli SR, Pabbaraja S, Vangala JR, Kalivendi SV. (**2011**) Stereoselective total synthesis of (+)-oploxyne A, (-)-oploxyne B, and their C-10 epimers and structure revision of natural oploxyne B, *The Journal of Organic Chemistry*, ***76***, 2568-2576
[22] Yang MC, Kwon HC, Kim Y-J, Lee KR, Yang HO. (**2010**) Oploxynes A and B, polyacetylenes from the stems of *Oplopanax elatus*. *Journal of Natural Products*, ***73***, 801-805.
[23] Satoh Y, Satoh M, Isobe K, Mohri K, Yoshida Y, Fujimoto Y. (**2007**) Studies on Panax acetylenes: Absolute structure of a new Panax acetylene, and inhibitory effects of related acetylenes on the growth of L-1210 cells, *Chemical & Pharmaceutical Bulletin (Tokyo)*, ***55***, 561-564
[24] Christensen LP, Brandt K. (**2006**) Bioactive polyacetylenes in food plants of the Apiaceae family: Occurrence, bioactivity and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, ***41***, 683-693.
[25] Christensen LP, Jakobsen HB. (**2008**) Polyacetylenes: distribution in higher plants, pharmacological effects and analysis. In *Thin Layer Chromatography in Phytochemistry*, Vol. ***99***, Waksmundzka-Hajnos M, Sherma J, Kowalska T. (Eds), CRC Press, Boca Raton, FL, USA
[26] Zidorn C, Johrer K, Ganzera M, Schubert B, Sigmund EM, Mader J, Greil R, Ellmerer EP, Stuppner H. (**2005**) Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. *Journal of Agricultural and Food Chemistry*, ***53***, 2518-2523.
[27] Søltoft M, Eriksen M, Träger A, Nielsen J, Laursen K, Husted S, Halekoh U, Knuthsen P. (**2010**) Comparison of polyacetylene content in organically and conventionally grown carrots using a fast ultrasonic liquid extraction method, *Journal of Agricultural and Food Chemistry*, ***58***, 7673-7679