

## Bioassay-directed isolation of falcarindiol and isoacetovanillon from *Pycnocycla caespitosa* based on KCl-induced contraction in rat uterus smooth muscles

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### Abstract

Hydroalcoholic extract and essential oil of aerial parts of *Pycnocycla caespitosa* have spasmolytic activity on rat ileum contractions. The objective of this research was to separate fractions of total hydroalcoholic extract of *P. caespitosa* guided by their spasmolytic activity on rat uterus. Aerial parts of *P. caespitosa* were extracted with ethanol. The concentrated extract was subjected to column chromatography and thin layer chromatography (TLC) for isolation fractions, then one of the bioactive fractions was subjected to further isolation to find its active components. Five fractions were obtained (Fr.1-Fr.5) and their anti-spasmodic activities were examined on uterus contraction induced by KCl (80 mM) and compared with ritodrine. In addition, spasmolytic effect of Fr.4 (one of the bioactive fractions) was determined on rat uterus induced by oxytocin (0.0005 IU/mL) and compared with ritodrine. Hydroalcoholic extract of *P. caespitosa* (0.032-2 mg/mL) reduced the responses to KCl but the inhibitory effect was not complete with 2 mg/mL extract in the bath. Four fractions (Fr.1, Fr.2, Fr.3 and Fr.4) (32-500 µg/mL) inhibited rat uterus contractions on the uterus while Fr.4 was slightly more active than others (IC<sub>50</sub> = 146 ± 23 µg/mL). Falcarindiol and isoacetovanillone were identified from Fr.4 using phytochemical methods including high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and TLC. In conclusion, in this research bioactivity guided technique was successfully used for separation of active fraction of *P. caespitosa*. Falcarindiol and isoacetovanillone were identified from the active fraction which inhibited both tonic and rhythmic contractile responses in rat isolated uterus.

**Keywords:** Isoacetovanillone; Falcarindiol; *Pycnocycla caespitosa*; Uterus; Bioactivity; Anti-spasmodic

### INTRODUCTION

*Pycnocycla caespitosa* Boiss. & Hausskn belongs to genus of *Pycnocycla*, subfamily of Umbellales and family of Umbelliferae (1). *Pycnocycla* contains eight different species in Iran from which *P. spinosa* phytochemistry and pharmacological properties is published by the same authors in previous researches (2-8). Hydroalcoholic extract of *P. spinosa* has shown spasmolytic effect on ileum, uterus and bladder smooth muscles in vitro, antidiarrheal and spasmolytic activity in vivo (6-9). Using a bioassay-guided isolation from aerial parts of *P. spinosa*, a new polycyclic diterpenoid,

(3,7,10,14,15-pentaacetyl-5-butanoyl-13,17-epoxy-8-myrsinene), vanillin, isoacetovanillone, and a new phenolic compound, 6-(4-hydroxy-3-methoxyphenyl)-hexanoic acid with inhibitory effects on KCl-induced smooth muscle contractions on the rat were isolated (8-11). It is likely that other species of *Pycnocycla* have similar pharmacological activities like spasmolytic effect on uterus. *P. caespitosa* naturally grows in some parts of Kohgiluyeh and Boyer-Ahmad and Chaharmahal and Kohgiluyeh provinces of Iran (12).

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In Tangesolakin Kohkiloyah and Boyerahmed this plant is known as BonjehKharo and its boiled extract is traditionally used as a drug for reducing dysmenorrhea. Its extract has anti-inflammatory and analgesic effects in animal model of inflammation and pain (13,14). The main components of its essential oil were identified as carvacrol,  $\beta$ -eudesmol,  $p$ -cymene, caryophyllene oxide,  $\alpha$ -pinine and  $\alpha$ -phelandrene (15). The hydroalcoholic extract and essential oil of *P. caespitosa* inhibit ileum contraction induced by various spasmogens including acetylcholine, electrical field stimulation and KCl, *in vitro* (15). However, the hydroalcoholic extract of *P. caespitosa* is a mixture of unknown substances with different pharmacological activities. Therefore, it is essential the active substances that are relaxant of uterus smooth muscle be identified.

## MATERIALS AND METHODS

### Drugs and solutions

Solidified extract or fractions (Fr.1 – Fr.5) were weighed and prepared as 20 or 40 mg/mL stock solution in dimethyl sulfoxide (DMSO). Ritodrine (Spain; prepar®) was prepared as 10 mg/mL stock solution in distilled water. Further dilutions were made in distilled water. KCl was prepared as 2 M stock solution. Oxytocin ampule (Aburaihan Pharm., Iran) was prepared as 10 IU/mL stock solution and diluted to 1 IU/mL solution with distilled water. 17- $\beta$ -estradiol was prepared as 100  $\mu$ g/mL stock solution in edible oil. Tyrode's solution (NaCl: 136.9, KCl: 2.68, CaCl<sub>2</sub>: 1.8, MgCl<sub>2</sub>: 1.05, NaHCO<sub>3</sub>: 11.9, NaH<sub>2</sub>PO<sub>4</sub>: 0.42, and glucose: 5.55 (mM)), was prepared in distilled water. Unless stated, all the chemicals were purchased from Merck Company (Germany).

### Plant materials

Aerial parts of *P. caespitosa* Boiss. & Hausskn (umbelliferae) were collected in June, 2015 from Gachsaran (Kohgiloye and Boyerahmad province, Iran). It was identified and compared with voucher specimen of the plant (No. 3042) deposited in the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Iran.

### Extraction and fractionation

Dried plant material (5 kg) was macerated with 40 L ethanol for three days and repeated three times at room temperature, and then concentrated under reduced pressure using a rotary evaporator. The yielded gummy extract was loaded on a vacuum liquid chromatography (VLC) charged with reverse silica-gel packed into a Büchner funnel with a sintered glass disc (150 × 90 mm) using MeOH: H<sub>2</sub>O as eluent. Washing VLC with MeOH: H<sub>2</sub>O (7:3) eluted a semi polar fraction (390 g light brown filtrate) and retained polar constituents. Defatted fraction was chromatographed on normal open column using gradient mixtures of hexane: acetone and yielded five fractions: Fr.1 (90:10); Fr.2 (85:15); Fr.3 (80:20); Fr.4 (70:30); Fr. 5 (0:100). Fractions were concentrated to dryness and stored at 0 °C until use.

### Uterine contractile assessment

Experiments were conducted on adult non-pregnant female Wistar rats (180-250 g) bred in School of Pharmacy animal house. All animals were handled in compliance with the principles of the guide for care and use of laboratory animal care. A day before experiment, rats were pretreated with 17- $\beta$ -estradiol (100  $\mu$ g/kg, SC), and housed in cages with free access to food and water at room temperature. On the day of experiment, rats were killed by a blow on the head, followed by exsanguination. Both uterine horns were removed and placed in oxygenated Tyrode's solution at room temperature. The uterine horns were separated from each other. A section of uterine horn was mounted for isotonic contraction under 1 g tension in 20 mL organ bath (Harvard, England) containing Tyrode's solution and continuously gassed with O<sub>2</sub> at 37 °C. Uterine contraction was measured using a Harvard isotonic transducer and recorded on a Harvard Universal Oscillograph (England) pen recorder device. The other uterine horn was used as vehicle treated time matched control tissue. After calibrating the oscillograph, 3 successive washes was given to the tissue and allowed to relax to a stable base line. Following a resting period of about 30 min, inhibitory effect of

*P. caespitosa* extract or fractions (Fr.1-Fr.5) were examined on isolated rat uterine contraction induced by KCl or oxytocin as described before (16) and compared with ritodrine as standard drug. All procedures were reviewed and approved by the university animal care committee.

#### **Effect of drugs on spasm induced by potassium chloride**

Uterine horns were exposed to KCl (80 mM) to induce tonic contraction. After 15 min equilibration time, drugs were added in a cumulative manner to the bath until maximum inhibition was obtained. The time matched control tissues were treated with equivalent volume of relaxant vehicle.

#### **Effect of drugs on spasm induced by oxytocin**

Rhythmic spasms of uterine were induced by adding oxytocin (0.0005 IU/mL) into the bath. After 5 min equilibration time, drugs were added to the bath in a cumulative manner until maximum inhibition was achieved. The time matched control tissues were treated with equivalent volume of relaxant vehicle.

#### **Isolation of bioactive compounds**

Relaxant effect of the resulted fractions: Fr.1 to Fr.5 were compared *in vitro* on KCl-induced contraction in rat uterus smooth muscles. The most bioactive fraction, Fr.4 was selected and subjected on a sepak RP-18 cartridge using methanol:water (7:3) as mobile phase to remove chlorophylls and fats. Defatted fraction was injected to high-performance liquid chromatography (HPLC) using YMC-Pak-Sil column (250 × 20 mm) and hexane/EtOAc (80:20) as mobile phase to yield compounds **1** and **2** as the major bioactive compounds.

Compound **1**: pale oil. IR (NaCl)  $\nu_{\max}$ : 3354, 3024, 2964, 2939, 2860, 2360, 2254, 2152, 1647, 1560, 1468, 1419, 1379, 1304, 1269, 1120, 1020, 987, 933, 879  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz, J in Hz):  $\delta_{\text{H}}$  0.90 (3H, t, J = 6.8 Hz, H3-17), 1.29 (10H, m, H2-12, 13, 14, 15, 16), 2.01 (2H, q, J = 7.2 Hz, H2-11), 2.41 (2H, br s, 3-OH and 8-OH), 4.96 (1H, d, J = 4.8, H-3), 5.21 (1H, d, J = 8.0, H-8), 5.27 (1H, d, J = 10, H-1b), 5.46 (1H, d,

J = 16.8, H-1a), 5.53 (1H, dd, J = 9.2, 8.0, H-9), 5.64 (1H, dt, J = 9.2, 7.2, H-10), 5.97 (1H, ddd, J = 5.2, 10.0, 16.8 Hz, H-2).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz):  $\delta_{\text{C}}$  14.1 (C-17), 22.6 (C-16), 27.7 (C-15), 29.11, 29.16, 29.3 (C-12, C-13, C-14), 31.8 (C-11), 58.6 (C-8), 63.4 (C-3), 68.7 (C-5), 70.3 (C-6), 78.3 (C-4), 79.9 (C-7), 117.3 (C-1), 127.7 (C-10), 134.6 (C-9), 135.8 (C-2). EI-MS m/z 259 [M-H].

Compound **2**: white solid;  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $^1\text{H-NMR}$  (400 MHz) 7.47 (2H, m, H-2, H-6), 6.88 (1H, d, J = 8.7, H-5), 3.89 (3H, s, 4-OMe), 2.49 (3H, s, 2'-Me);  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ): 26.21 (C-2'), 56.08 (4-OMe), 109.69 (C-5), 113.75 (C-2), 124.02 (C-6), 130.24 (C-1), 146.60 (C-3), 150.39 (C-4), 196.83 (C-1'); EI-MS (m/z): 166 [M].

#### **Data analysis**

The contractile response to KCl and oxytocin were measured as maximum amplitude from pretreatment baseline and expressed as the percentage of the initial response in the absence of drugs or vehicle for each tissue. All the values are quoted as mean  $\pm$  standard error of the mean (SEM). The  $\text{IC}_{50}$  value (drug concentration causing 50% of maximum response) of the relaxant uterus was calculated for each tissue and mean and SEM was determined for each group of results. Sigma plot computer program was used for statistical analysis and drawing the graphs for calculation of  $\text{IC}_{50}$  values.

## **RESULTS**

#### **Effects on KCl-induced smooth muscle contraction in rat uterine**

KCl (80 mM) caused a sustained tonic contraction which maintained for the duration of study. The hydroalcoholic extract of *P. caespitosa* (32  $\mu\text{g/mL}$ -2  $\text{mg/mL}$ ) reduced the responses to KCl but the inhibitory effect was not complete and with bath concentration of 2  $\text{mg/mL}$  the KCl response was only inhibited by 48%. Fractions Fr.1, Fr.2, Fr.3 and Fr.4 (32-500  $\mu\text{g/mL}$ ) were bioactive fractions and 100% inhibition of them was achieved at concentration of 500  $\mu\text{g/mL}$  in the bath. Fraction 5 had no antispasmodic activity. Ritodrine (5-320  $\mu\text{g/mL}$ ) in a similar way

inhibited tonic contraction induced by KCl. In detail the  $IC_{50}$  found against KCl-induced contractions were (Fr.1)  $155 \pm 23$ ; (Fr.2)  $197 \pm 25$ ; (Fr.3)  $171 \pm 35$ ; (Fr.4)  $146 \pm 24$   $\mu\text{g/mL}$  while ritodrine were  $153 \pm 20$   $\mu\text{g/mL}$ . Fr.4 was slightly more active than others (Fig. 1). The  $IC_{50}$  values are compared in Table 1.

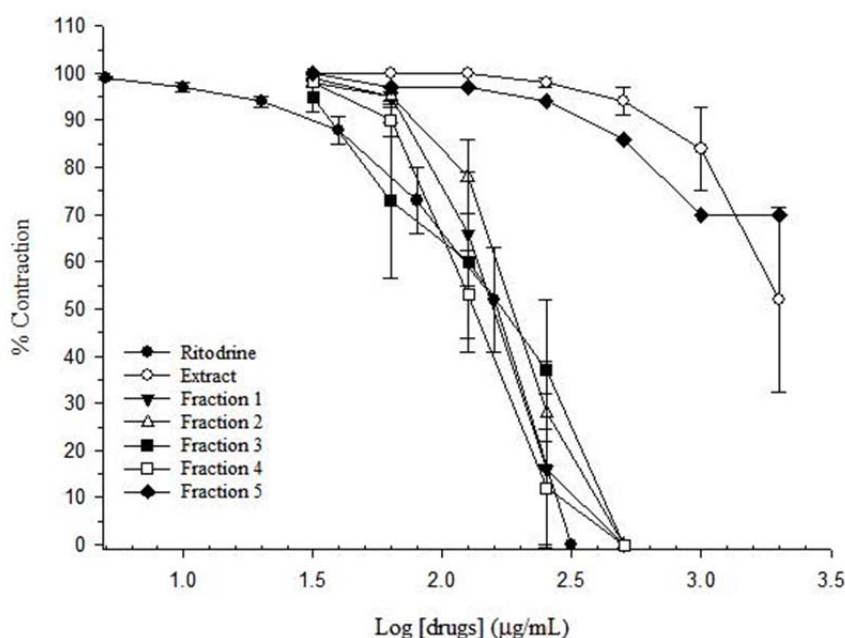
#### Effects on oxytocin -induced smooth muscle contraction in rat uterine

Oxytocin (0.0005 IU/mL), caused a relatively regular rhythmic contraction on rat uterus. Fr.4 (32-1000  $\mu\text{g/mL}$ ), and ritodrine (5-1280  $\mu\text{g/mL}$ ) concentration-dependently inhibited the rhythmic contraction induced by oxytocin (Fig. 2). Antispasmodic activity of

Fr.4 was comparable with ritodrine with  $IC_{50}$  values of  $130 \pm 12$ , and  $140 \pm 48$   $\mu\text{g/mL}$ , respectively (Fig. 2). The  $IC_{50}$  values are compared in Table 1.

#### Identification of bioactive compounds

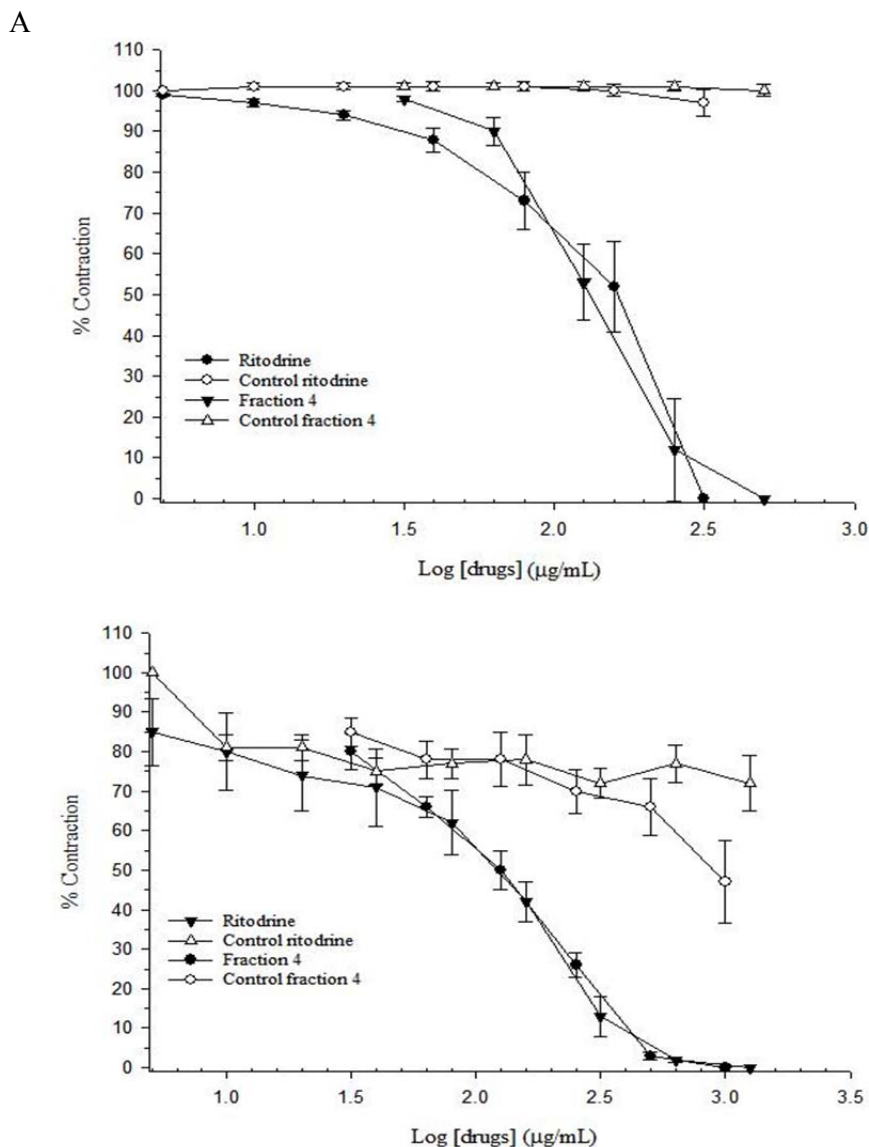
The ethanol extract of *P. caespitosa* was chromatographed on normal silica gel column by stepwise gradient elution from hexane to ethyl acetate. Using a bioassay-directed fractionation *in vitro* on KCl-induced contraction in rat uterus smooth muscles, the most active fraction, Fr.4 was subjected to more purification on recycle HPLC and yielded compounds **1** and **2** as the major bioactive compounds (Fig. 3).



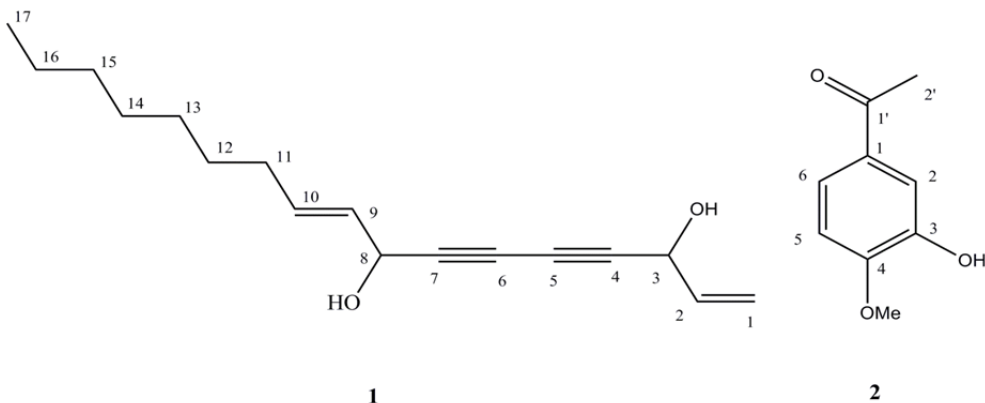
**Fig. 1.** Inhibitory effects of *Pycnocycla caespitosa* extract and its fractions (Fr.1-Fr.5) on tonic contractions developed in rat isolated uterus by KCl (80 mM). Contractile response was measured relative to the baseline. Ordinate scales: spasm remaining as a percentage of the contraction prior to drugs addition. Abscissa scales:  $\log_{10}$  concentration of compounds. Each point is mean of six experiments and the vertical lines show the SEM ( $n = 6$ ). The maximum concentration of DMSO in the bath was 5%.

**Table 1.** Comparison of the  $IC_{50}$  values ( $\pm$  SEM) of *P. caespitosa* fractions (Fr.1, Fr.2, Fr.3 and Fr.4), ritodrine on contraction induced by KCl and oxytocin in rat isolated uterus ( $n = 6$ ).

	KCl	Oxytocin
Fr.1	$155 \pm 23$ $\mu\text{g/mL}$	-
Fr.2	$197 \pm 25$ $\mu\text{g/mL}$	-
Fr.3	$171 \pm 35$ $\mu\text{g/mL}$	-
Fr.4	$146 \pm 24$ $\mu\text{g/mL}$	$130 \pm 12$ $\mu\text{g/mL}$
Ritodrine	$153 \pm 20$ $\mu\text{g/mL}$	$140 \pm 48$ $\mu\text{g/mL}$



**Fig. 2.** Comparison of inhibitory effect of Fr. 4 of *P. caespitosa* on tonic contractions developed in rat isolated uterus by (A) KCl, and (B) oxytocin (0.0005 IU/mL). Contractile response was measured relative to the baseline. Ordinate scales: spasm remaining as a percentage of the contraction prior to drugs addition. Abscissa scales:  $\log_{10}$  concentration of compounds. Each point is mean of six experiments and the vertical lines show the SEM (n = 6). The maximum concentration of DMSO in the bath was 5%.



**Fig. 3.** Compounds 1-2 isolated from antispasmodic fraction of *P. caespitosa*.

Compound **1**, was obtained as a pale oil with EI-MS molecular ion at  $m/z$  260 [M]. IR,  $^{13}\text{C}$ -NMR and DEPT spectra showed 17 carbons comprising of two olefin bonds including a vinyl group [ $\nu_{\text{max}}$  1647 and 987  $\text{cm}^{-1}$ ;  $\delta_{\text{C}}$  117.3 ( $\delta_{\text{H}}$  5.27, 1H, d,  $J = 10$ , H-1b; 5.46, 1H, d,  $J = 16.8$ , H-1a);  $\delta_{\text{C}}$  135.8 ( $\delta_{\text{H}}$  5.97, 1H, ddd,  $J = 5.2, 10.0, 16.8$  Hz, H-2)], and a disubstituted double bond [ $\delta_{\text{C}}$  134.6 ( $\delta_{\text{H}}$  1H, 5.53, dd,  $J = 8.0, 9.2$ , H-9), and 127.7 ( $\delta_{\text{H}}$  5.64, 1H, dt,  $J = 9.2, 7.2$ , H-10)], two disubstituted alkyne bonds [ $\nu_{\text{max}}$  2152, 2254  $\text{cm}^{-1}$ ;  $\delta_{\text{C}}$  68.7, 70.3, 77.3, 79.9], two hydroxymethine groups [ $\nu_{\text{max}}$  3354  $\text{cm}^{-1}$ ;  $\delta_{\text{C}}$  63.4 ( $\delta_{\text{H}}$  4.96, 1H, d,  $J = 4.8$ , H-3), 58.6 ( $\delta_{\text{H}}$  5.21, 1H, d,  $J = 8.0$ , H-8)], six methylenes ( $\delta_{\text{C}}$  31.8, 29.3, 29.2, 29.1, 27.7, 22.6) and one methyl carbon [ $\delta_{\text{C}}$  14.1 ( $\delta_{\text{H}}$  0.90, 3H, t,  $J = 6.8$  Hz, H<sub>3</sub>-17)]. Application of  $^1\text{H}$ - $^1\text{H}$  correlation spectroscopy (COSY) allowed detecting two spin systems of H-1 to H-3: CH<sub>2</sub>=CH-CHOH and H-8 to H-10: CHOH-CH=CH-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub> and assigned the hydroxymethines signals at C-1 and C-9. Finally, hydroxymethine protons at  $\delta_{\text{H}}$  4.96 (H-3) and 5.21 (H-8) with HMBC cross-links with alkyne carbons, besides the fragments  $m/z$  105 in the ESI-MS (fragment C-1 to C-7) assigned alkyne carbons at C-4, C-5, C-6, and C-7. These structural feature were similar to those of heptadeca-1,9-dien-4,6-diyne-3,8-diol named as falcarindiol but with a new and more precise assignment (17). Besides falcarindiol, a minor impurity with acetylen structure and similar IR,  $^1\text{H}$ -NMR and mass fragmentation pattern was identified differed with **1** in presence of a carbonyl group [ $\nu_{\text{max}}$  1716  $\text{cm}^{-1}$ ], loss of two protons in molecular ion  $m/z$  258 in EI-mass spectrum,  $^1\text{H}$ -NMR downfield shifts of H-1a: 5.96 (1H, d,  $J = 11.1$ Hz), H1b: 5.70 (1H, d,  $J = 15.3$  Hz), H-2: 6.52 (1H, dd,  $J = 11.1, 15.3$ Hz) and lack of hydroxymethine H-3 resonance (see experimental). These structural feature were similar to those of heptadeca-1,9-dien-4,6-diyne-8-ol-3-ene named as falcarinolon and seems to be produced as an artifact by autooxidation of compound **1** (18).

Compound **2** was obtained as a white solid with positive reaction to methanolic ferric chloride reagent and molecular ion  $m/z$  166 in

EI-mass spectrum.  $^1\text{H}$ -NMR spectrum showed ABX spin pattern similar to trisubstituted vanillin derivatives at  $\delta_{\text{H}}$  7.47 (1H, d,  $J = 2.0$  Hz, H-2), 7.47 (1H, dd,  $J = 8.7, 2.0$  Hz, H-6), 6.88 (1H, d,  $J = 8.7$  Hz, H-5), one methoxy at  $\delta_{\text{H}}$  3.89 (3H, s), and one downfield methyl singlet at  $\delta_{\text{H}}$  2.49 (3H, s). These data were similar to those reported for isoacetovanillone which was more confirmed by co-TLC with standard (19).

## DISCUSSION

*P. caespitosa* extract is a relaxant of isolated uterus and in the current study we have shown that its inhibitory effect on rat uterus starts with about 256  $\mu\text{g}/\text{mL}$  extract in the bath and with bath concentration of 2  $\text{mg}/\text{mL}$  48% of the responses to KCl was inhibited. Administration of active components rather than the total extract has two main advantages.

Firstly, the relative purity is increased and it is more feasible to give a standard dose. Secondly, administration of inactive substances is avoided.

The main objective of the present study was to isolate the active fractions of *P. caespitosa* extract and to compare it with the total extract. The main problem was that we had no idea about the chemical properties of the components exist in the extract. Therefore, we have used a bioassay technique based on the bioactivity properties of the separated components of the *P. caespitosa* extract. In the initial phase of separation, we have separated inactive fractions from active fractions. Four active fractions were identified which totally inhibited the contractile response to KCl and were more potent than the total extract. Fr.4 at similar ranges of concentrations also inhibited the periodic contraction on rat uterus induced by oxytocin (Table 1).

The inhibitory concentration ranges of isolated active fraction of *P. caespitosa* was similar with that of ritodrine. Ritodrine by activating  $\beta_2$ -adrenoceptor increases adenylyl cyclase activity and production of intracellular cAMP which inhibits contractile proteins in smooth muscles (21).

As Fr.4 was slightly more active than other fractions and therefore, subjected to further separation and identification of its components using HPLC analysis. Falcarindiol and isoacetovanillone were identified as main constituents. In addition two minor constituents with acetylene structures existed which were not identified. Isoacetovanillone is also identified as one of the active component of *P. spinosa* extract and has been reported to have relaxant effect on ileum contractions (21). In another study by Matsuda, *et al.* in a model of norepinephrine and KCl-induced contractions on isolated thoracic aorta of rats, falcarindiol isolated from *Angelica furcijunga* (Umbelliferae) nonselectively inhibited both contractions (22).

### CONCLUSION

In this research bioactivity guided technique was successively used for separation of active fraction of *P. caespitosa*. The more active fraction inhibited both tonic and rhythmic contractile responses in rat isolated uterus. Falcarindiol and isoacetovanillone are two component which were identified in the active fraction. Therefore, further research on these two component are suggested.

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### REFERENCES

- Mozaffarian V. The family of Umbellifera in Iran. Tehran: Research Institute of Forests and Rangelands; 1983. pp. 1-101.
- Sadraei H, Asghari G, Naddafi A. Relaxant effect of essential oil and hydroalcoholic extract of *Pycnocycla spinosa* Decne. exBoiss on ileum contractions. *Phytother Res.* 2003;17:645–649.
- Sadraei H, Asghari G, poorkhosravi R. Spasmolytic effect of root and aerial parts extract of *Pycnocycla spinosa* on neural stimulation of rat ileum. *Res Pharm Sci.* 2011;6(1):43–50.
- Sadraei H, Asghari G, Hekmatti AA. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa*. *J Ethnopharmacol.* 2003;86:187–190.
- Sadraei H, Asghari G, Andisha M. Antispasmodic effect of *Pycnocycla spinosa* seed and aerial part extract on rat ileum and uterus smooth muscle contractions. *Daru.* 2008;13:160-163.
- Sadraei H, Asghari G, Arabzadah A. Effect of hydroalcoholic extract of *Pycnocycla spinosa* on rat isolated bladder. *Iranian J Pharm Res.* 2004;4:237-241.
- Sadraei H, Asghari G, Shams M. Antidiarrheal action of hydroalcoholic extract of *Pycnocycla spinosa* in comparison with loperamide and dicyclomine. *Iran J Pharm Res.* 2011;10:835–841.
- Sadraei H, Asghari G, Behzad S. Bioactivity-guided isolation of spasmolytic components of *Pycnocycla spinosa* Decne exBoiss. *Res Pharm Sci.* 2011;6: 81-86.
- Ghanadian M, Sadraei H, Yousuf S, Asghari G, Choudhary MI, Jahed M. New diterpene polyester and phenolic compounds from *Pycnocycla spinosa* Decne. Ex Boiss with relaxant effects on KCl-induced contraction in rat ileum. *Phytochemistry Letters.* 2014;7:57-61.
- Sadraei H, Ghanadian M, Asghari G, Sharifian R. 3,7,10,14,15 pentaacetyl-5-butanoyl-13,17-epoxy-8-myrsinene a novel compound isolated from *Pycnocycla spinosa* extract with potent antispasmodic and antidiarrheal properties. *Res Pharm Sci.* 2015;10:55-61.
- Sadraei H, Ghanadian M, Asghari G, Madadi E, Azali N. Antispasmodic and antidiarrheal activities of 6-(4-hydroxy-3-methoxyphenyl)-hexanoic acid from *Pycnocycla spinosa* Decne ex.Boiss. *Res Pharm Sci.* 2014;9:279-286.
- Parsa A. Flora of Iran. Tehran: Tehran University (Place holder 1) Publications; 1960. pp. 783.
- Arazi A, Azemi M, Fakhri A. Analgesic effect of hydroalcoholic extract of *Pycnocycla caespitosa* in rat by formalin test. *Jundishapur J Pharm.* 2003;5:105-113.
- Khodaei M. Assessment effect of hydroalcoholic extract of *Pycnocycla caespitosa* on karajyan inflammation in male paws rat. Pharm-D Thesis. [In Persian]. Jundishapur University of Medical Sciences. 2012. Identification number. 23668.
- Sadraei H, Asghari G, Alipour M. Anti-spasmodic assessment of hydroalcoholic extract and essential oil of aerial part of *Pycnocycla caespitosa* Boiss. & Hausskn on rat ileum contractions. *Res Pharm Sci.* 2016;11(1):33-42.
- Sadraei H, Shokoohinia Y, Sajjadi SE, Ghadirian B. Antispasmodic effect of osthole and *Prangos ferulacea* extract on rat uterus smooth muscle motility. *Res Pharm Sci.* 2012;7:141-149.
- Villegas M, Vargas D, Msonthi JD, Marston A, Hostettmann K. Isolation of the antifungal compounds falcarindiol and sarisan from *Heteromorpha Trifoliata*. *Planta Medica.* 1988;54:36-37.
- Bentley RK, Bhattacharjee D, Jones ER, Thaller V. Natural acetylenes. Part XXVIII. C 17-polyacetylenic alcohols from the *Umbellifer Daucus carota* L.(carrot): alkylation of benzene by

- acetylenyl (vinyl) carbinols in the presence of toluene-p-sulphonic acid. J Chem Soc C. 1969;4:685-688.
19. Fielding L, McKellar SC, Florence AJ. Precision studies in supramolecular chemistry: a  $^1\text{H}$ -NMR study of hydroxymethoxyacetophenone/ $\beta$ -cyclodextrin complexes. Magnetic Resonance Chem. 2011;49:405-412.
  20. Ikeda S, Tamaoki H. Pharmacological investigation of ritodrine hydrochloride, a  $\beta$ 2-adrenoceptor stimulant. Jpn J Pharmacol. 1984;36(4):477-484.
  21. Sadraei H, Ghanadian M, Asghari G, Madadi E. Antispasmodic activity of isovanillin and isoacetovanillon comparison with *Pycnocyclus spinosa* Decne. exBoiss extract on rat ileum. Res Pharm Sci. 2014;9:187-192.
  22. Matsuda H, Murakami T, Nishida N, Kageura T, Yoshikawa M. Vasorelaxant active constituents from the roots of *Angelica furcijuga* Kitagawa: structures of hyuganins A, B, C, and D. Chem Pharmaceut Bulletin. 2000;48:1429-1435.