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Aktivitas trakheospasmolitik vitetrifolin-E yang diisolasi dari daun *Vitex trifolia* L.

Tracheospasmolytic activity of vitetrifolin-E isolated from the leaves of *Vitex trifolia* L.

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Abstrak

Dalam upaya melanjutkan pencarian senyawa aktif trakheospasmolitik dari daun *Vitex trifolia*, sekarang kita laporkan isolasi dan identifikasi vitetrifolin E dari fraksi aktif yang diperoleh dari ekstrak n-heksana dengan metoda *Bioassay guided isolation*. Vitetrifolin-E mampu menghambat kontraksi spontan dari trakhea marmut jantan terisolasi hasil induksi dengan histamin, dan senyawa ini juga aktif pada trakhea marmut terisolasi tersensitisasi ovalbumin. Vitetrifolin-E menghambat kontraksi trakea sebesar 22,6% pada dosis pemberian 1,3 x 10^{-4} M dan 86,1% pada dosis 4 x10^{-4} M . Pada trakhea marmut tersensitisasi Vitetrifolin-E juga menghambat kontraksi sebesar 83,5% (1.3 x 10^{-5} M*).*
Kata kunci: vitetrifolin-E*, Vitex trifolia,* trakheospasmolitik

Abstract

In searching of tracheospasmolytic active compounds from *Vitex trifolia*, we are now reporting isolation and identification of vitetrifolin-E from active fraction obtained from n-hexane extract of *V. trifolia*. Vitetrifolin-E blocked spontaneous contraction of male guinea pig trachea induced by histamine and was active in a model using sensitized guinea pig trachea stimulated by ovalbumin. Vitetrifolin-E inhibited tracheal contraction by 22.6% at dose of 1.3 x 10^{-4} M and by 86.1% at dose of 4.10^{-4} M. In a model using sensitized male guinea pig trachea, vitetrifolin-E also inhibited the contraction 83.5% (1.3 x 10^{-5} M).
Keywords: vitetrifolin-E, *Vitex trifolia*, tracheospasmolytic

Introduction

In our continued search for tracheospasmolytic active natural products from Indonesian traditional medicines, we found that n-hexane extract of the leaves of *Vitex trifolia* was the interest of our study (Wahyuono *et al.* ,2000). At our previous report, 2 major tracheospasmolytic active compounds (viteosin A and vitexicarpin) were isolated from active fraction obtained by bioassay-guided fractionation (Alam *et al.*, 2002). At present, we are reporting isolation of other tracheospasmolytic active compound

from similar active fraction and we identified the compound as vitetrifolin-E.

Methodology

Material

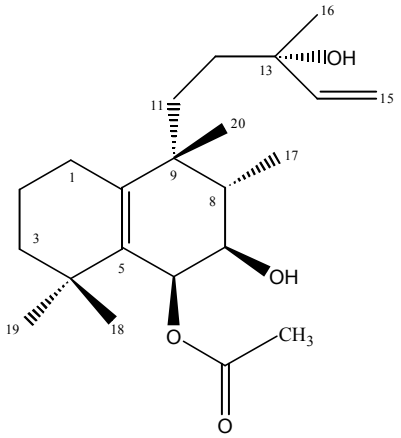
The leaves of *V. trifolia* were collected from the plantation of the Medicinal Plant Research Center (BPTO), Tawangmangu, Central Java (Indonesia) in December 1998. A voucher specimen (BF 801, code VT) was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta (Indonesia). Vitetrifolin-E was isolated from the active fraction obtained by tracheospasmolytic bioassay-guided solvent extraction and partition of the n-hexane extract of *V. trifolia* described in the previous paper (Alam *et al*., 2002). The active fraction was triturated with 80% MeOH to give 80% MeOH insoluble and soluble fractions.

Isolation

Vitetrifolin-E, the major compound presence in the 80% MeOH soluble fraction was purified by preparative TLC [SiO_2 GF_{254} E. Merck ($CHCl_3$-EtOAc, 6:1 v/v)]. Vitetrifolin-E appeared as a homogenous spot on TLC [Rf. 0.14 ($CHCl_3$-EtOAc, 6:1 v/v), Rf. 0.52 ($CHCl_3$-EtOAc, 6:3 v/v)]. The structure of vitetrifolin-E was determined by UV, IR, Mass and NMR spectra data. The UV spectrum was measured on a Cary 1Bio UV-Visible spectrometer, the IR spectrum was obtained using a PYE-UNICAM SP_3-200 spectrometer, the mass spectrum was obtained from Finnigan MAT TSQ-700 triple quadrupole equipped with a costum-made Electro Spray Interface (ESI) and the NMR spectra were taken on a Bruker DPX-300 spectrometer. The melting By means of partition and preparative TLC, vitetrifolin-E (**1**) [(6S, 7R, 8S, 9R)-6-acetoxy-5(10),14-halimadien-7,13-diol] was isolated from the active fraction and identified on the basis of its spectroscopic and reported data. Vitetrifolin-E (dose 1.3 x 10^{-4} M) inhibited the tracheal contraction by 22.6%, and when the dose was raised to 4.0 x 10^{-4} M the tracheal contraction was inhibited by 86.1% (Fig. 1). Compared to viteosin-A and vitexicarpin, tracheospasmolytic activity of vitetrifolin-E (4.0 x 10^{-4} M) was in between of those of viteosin-A and vitexicarpin. In our previous report [*Alam et.at*, 2002], viteosin-A point (m.p., uncorrected) was taken from Reichert, Austria.

Tracheospasmolytic test

The male guinea pigs (350-550 g) were obtained from Laboratory of Animal Development Unit, Gadjah Mada University, Yogyakarta, Indonesia. Vitetrifolin-E was evaluated for its ability to inhibit non-sensitized tracheal contraction induced by histamine and sensitized animal induced by ovalbumin. Each assay was conducted in a 20 ml organ bath filled with Krebs buffer solution (containing 3 x 10^{-6} M indomethacin), and tracheal contraction was induced by histamine (10^{-7}-10^{-3} M). Vitetrifolin-E (1.3 x 10^{-4} M and 4.0 x 10^{-4} M) was added to organ bath, incubated for 30 min, and tracheal contraction inhibition was measured using a Grass force-displacement transducer, model 388 connected to a Kipp & Zone polygraph BD 1. For tracheal sensitized guinea pigs, the animals were sensitized by 0.2 ml of ovalbumin (1 mg/ml), 3-4 weeks before the experiment. Tracheal contraction was sequentially induced by 5, 50, 500, and 5000 ng/ml ovalbumin, carbachol (10^{-5} M), and in saturated KCl, at 15 min intervals. The dose tested for vitetrifolin-E in the sensitized animal was 1.3 x 10^{-5} M, and DMSO (10 µl) as solvent was used as control (-). All the results are expressed as the mean of 3 experiments ± SD, and the tracheal contraction inhibition was calculated as (100 – response) (%).



VITETRIFOLIN-E

Results And Discussion

By means of partition and preparative TLC, vitetrifolin-E [(6S, 7R, 8S, 9R)-6-acetoxy-5(10),14-halimadien-7,13-diol] was isolated from the active fraction and identified on the basis of its spectroscopic and reported data (Ono *et al*, 2001). Vitetrifolin-E (dose 1.3 x 10^{-4} M) inhibited the tracheal contraction by 22.6%, and when the dose was raised to 4.0 x 10^{-4} M the tracheal contraction was inhibited by 86.1% (Fig. 1). Compared to viteosin-A and vitexicarpin, tracheospasmolytic activity of vitetrifolin-E (4.0 x 10^{-4} M) was in between of those of viteosin-A and vitexicarpin. In our previous report [2], viteosin-Aonly inhibited trachea contraction by 47.9%, and vitexicarpin displayed almost a complete inhibition

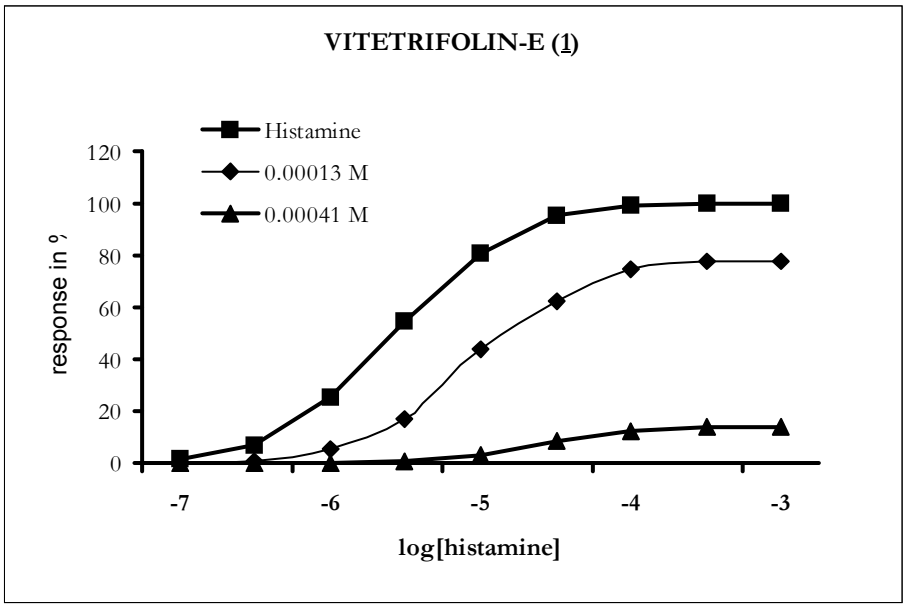


Figure 1. Log dose-response curve of vitetrifolin-E in the tracheospasmolytic test induced by histamine on non-sensitized male guinea pig

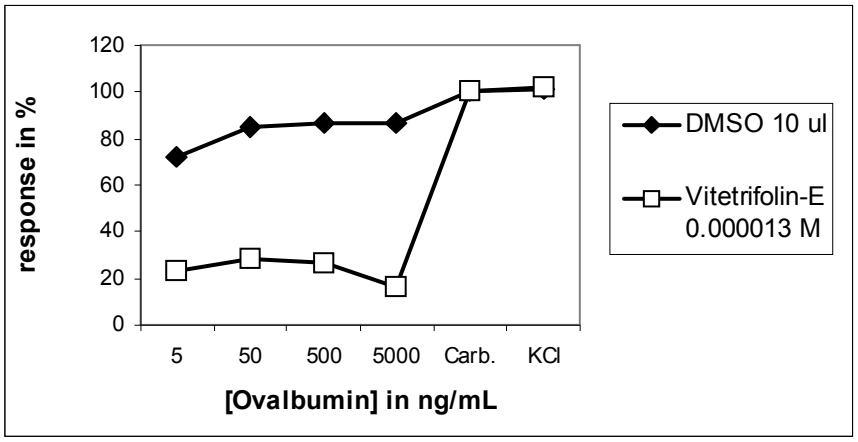


Figure 2. Tracheospasmolytic test of vitetrifolin-E on sensitized male guinea pig

(97.2%) at equal dose (4.0 x 10^{-4} M). Similarly, tracheal contraction inhibition of vitetrifolin-E is dose dependent and its mechanism of action is similar to viteosin-A and vitexicarpin as non-competitive antagonism to histamine.

Further test of vitetrifolin-E (1.3 x 10^{-5} M) on a model using the trachea from sensitized male guinea pigs indicated that vitretrifolin-E inhibited tracheal contraction (83.50 $\pm$ 0.50%) and its activity was in between of those of viteosin-A (26.34 $\pm$ 5.51%) and vitexicarpin (93.57 $\pm$ 0.60%) (Alam *et al.*, 2002). Vitetrifolin-E was not harmful to organ similarly showed by both viteosin-A and vitexicarpin (Fig. 2).

Vitetrifolin-E appeared as colorless needles in acetone, m.p. 134-136 ^{0}C, showing UV abs. $\lambda_{max.}$ (MeOH), 215, 241 nm. The IR spectrum (cm^{-1}, KBr) (Figure 3) showed at 3433 (-OH), 3010 (=C-H), 2929 (-CH_2), 2871 (-C-H), 1718 (-C=O), 1629 (-C=C-), 1369 (-C=C-), 1255 (-C-O-) (Figure 3) (Silverstein

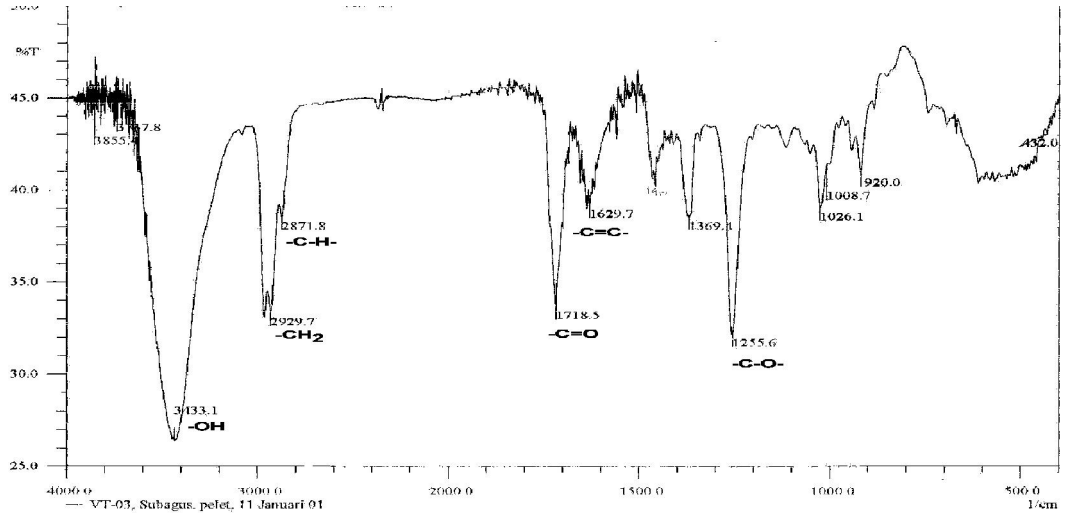


Figure 3. Infra red spectrum of vitetrifolin-E

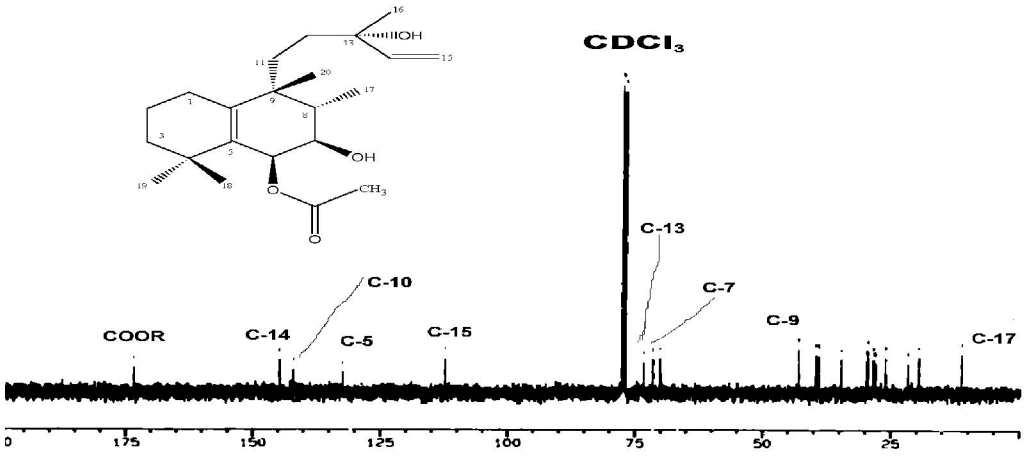


Figure 4. ^{13}C-NMR spectrum (75 MHz, $CDCl_3$) of vitetrifolin-E

Tabel I. The chemical shift of ^{13}C- and ^{1}H-NMR spectral data for vitetrifolin-E (400 MHz) compared with reference Ono *et al.*, 2001 (500 MHz) VT-03 = vitetrifolin-E.

No	^{1}H (Ref)	^{1}H VT-03	^{13}C- (Ref)	^{13}C-VT-03
1a	2.02	2.02	25.9	26.15
1b	2.02	2.02		
2a	1.64	1.62	19.5	19.7
2b	1.59	1.53		
3a	1.51	1.50	39.5	39.6
3b	1.47	1.47		
4			34.5	34.75
5			132.2	132.4
6	5.51 d (3.5)	5.5 d (3.2)	70.0	70.2
7	3.73 dd (3.5, 12.0)	3.71 dd (3.3, 12.2)	71.3	71.6
8	1.83 dq (12.0, 6.5)	1.83 dq (12.0, 6.5)	38.8	39.04
9			42.8	43.0
10			141.8	142.0
11a	1.44	1.42	29.5	29.76
11b	1.36 m	1.36		
12a	1.42	1.46	38.9	39.08
12b	1.11 m	1.11		
13			73.1	73.4
14	5.84 dd (11.0, 17.5)	5.81 dd	144.6	144.8
15a	5.18 dd (1.5, 17.5)	5.18 d	112.1	112.4
15b	5.06 dd (1.5, 11.0)	5.06 d		
16	1.24 s	1.23 s	28.1	28.3
17a	1.02 d (6.5)	1.01 d	11.1	11.29
18	1.05 s	1.04 s	29.3	29.56
19	0.94 s	0.92 s	27.8	28.48
20	1.05 s	1.03 s	28.3	28.05
COC	2.07 s	2.06 s	21.6	21.84
CO-			173.2	173.4

and Webster, 1997). ESIMS showed an $[M+Cl]^-$ at m/z 399 then the mol. weight was 364 and determined as $C_{22}H_{36}O_4$ by high-resolution positive ESIMS, the ^{13}C-NMR spectral data (300 MHz, $CDCl_3$, δ, ppm) (Figure 4) (Table 1) of vitetrifolin-E displayed 22 carbons signals with an ester signal (δ, 173.4 ppm) at the most down field signal in the spectra. Some distinguished signals were observed such as a terminal methylene (112.4), 3 other unsaturated carbons (132.4; 142.0 and 144.8) signals. Two present of alcoholic carbons were showed by signals at δ 73.4 and 71.6 ppm that were further identified as tertiary and secondary carbons by 2D-NMR techniques. The ^{1}H-NMR (300 MHz,

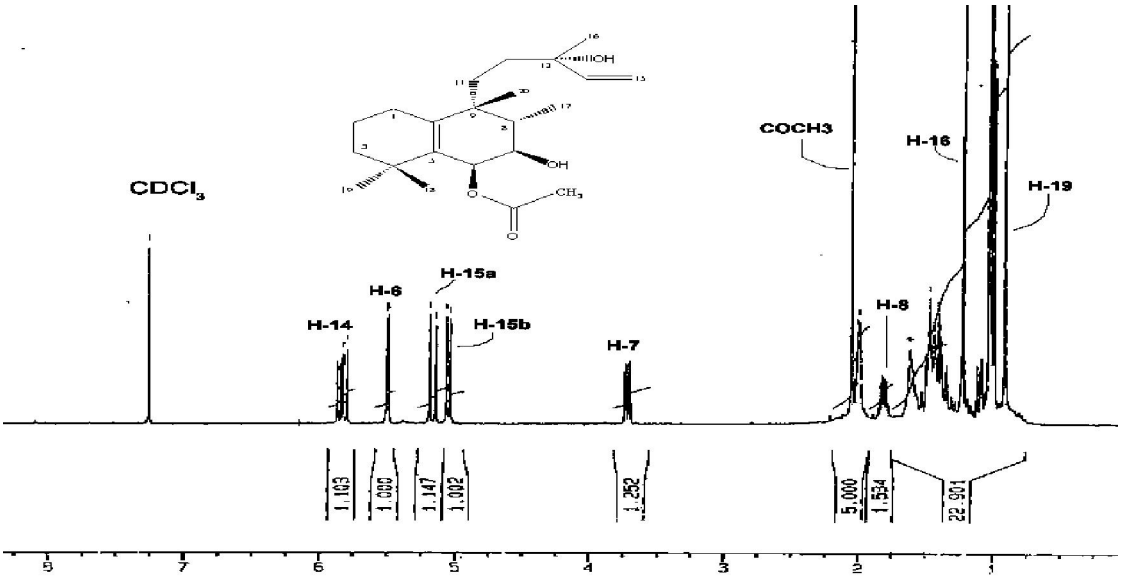


Figure 5. ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of vitetrifolin-E

$CDCl_3$, δ, ppm) (Tabel 1) (Figure 5) spectral data of vitetrifolin-E displayed olefinic signals at δ 5.84, 5.18 and 5.06 ppm integrated 1 proton each. COSY data showed that signals at 5.06 and 5.18 split each other and attached to the same carbon, therefore this data indicated these signals were protons of the terminal methylene. Signal at δ, 5.84 ppm (dd, 1H, 11.0, 17.5 Hz) displayed correlation (NOESY) to signals of the terminal methylene protons suggested that this signal corresponds to a proton of –CH=. There were 2 proton signals of –O-CH- (δ, 5.5 ppm, d, 3.2 Hz; 3.7 ppm, dd, 3.3 and 12.2 Hz) observed, signal at δ 5.5 was more downfield than it should be as this proton was in between adjacent to –C=C- and –OH groups. Five methyl signals were observed at the δ, 1.23-1.03 ppm, 4 of them appeared as singlet signals and the other was a doublet signal (δ, 1.01 ppm, J= 6.5 Hz). In addition, a methyl signal at δ, 2.06 ppm (s) was observed that was characteristic to a methyl signal of CH_3-C=O (acetyl) group (Paudler, 1987). Based on the data above and the reported spectroscopic data of vitetrifolin-E, this compound was confirmed as vitetrifolin-E (Ono *et al.*, 2001).

Conclusion

The active Compound was confirmed as Vitetrifolin-E. Vitetrifolin-E inhibited tracheal contraction by 22.6% at dose 1.3 x 10^{-4} M and by 86.1% at dose 4.10^{-4} M. In a model using sensitized male guinea pig trachea, vitetrifolin-E also inhibited the contraction 83.5% at dose 1.3 x 10^{-5} M.

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