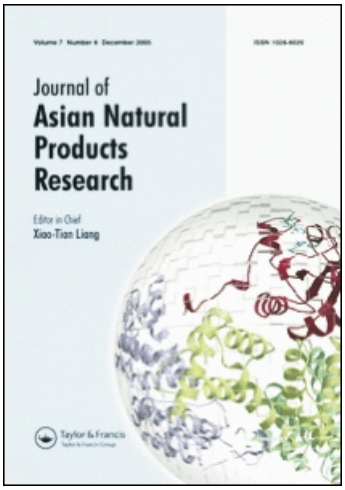


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# Synthesis and bioactivity of novel caffeic acid esters from *Zuccagnia punctata*

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# Synthesis and bioactivity of novel caffeic acid esters from *Zuccagnia punctata*

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Synthesis of novel caffeic acid esters (**1** and **2**) was accomplished starting from appropriately substituted benzaldehydes (**3** and **9**). While compound **2** exhibited potent anti-oxidative activity in both the nitroblue tetrazolium and 1,1-diphenyl-2-picrylhydrazyl radical-scavenging models, compound **1** showed moderate 5-lipoxygenase inhibitory activity.

*Keywords*: Zuccagnia punctata; Synthesis; Anti-oxidative; 5-Lipoxygenase

## 1. Introduction

Hydroxy substituted cinnamic acid esters are widely distributed in the plant kingdom and usually exist as esters of organic acids or sugars, or are bound to a protein and other cell wall polymers. The presence of such compounds in food significantly affects stability, colour, flavour, nutritional value and other food qualities [1]. Caffeic acid and its esters exhibit a wide range of pharmacological activities such as anti-oxidative [2–4], enzyme inhibitory [5–7], cytotoxic [8,9], antimicrobial [10], anti-allergy [11] and antiviral activities [12].

Svetaz et al. [13] recently isolated two novel caffeic acid ester derivatives, namely, 1′-methyl-3′-(4-hydroxyphenyl)propyl caffeate (**1**) and 1′-methyl-3′-(3,4-dihydroxyphenyl)-propyl caffeate (**2**) from *Zuccagnia punctata* and reported the antifungal activity of **1** against *Phomopsis longicolla*. As a part of our studies on the hydroxycinnamic acid esters [14,15], we report in this paper the synthesis and bioactivity studies on the caffeic acid esters, **1** and **2**, for the first time.

## 2. Results and discussion

Claisen-Schimdt condensation [16] of 4-hydroxybenzaldehyde (**3**) with acetone gave 4-(4-hydroxyphenyl)-3-buten-2-one (**4**) in 83% yield. Hydrogenation of **4** using hydrogen, in the

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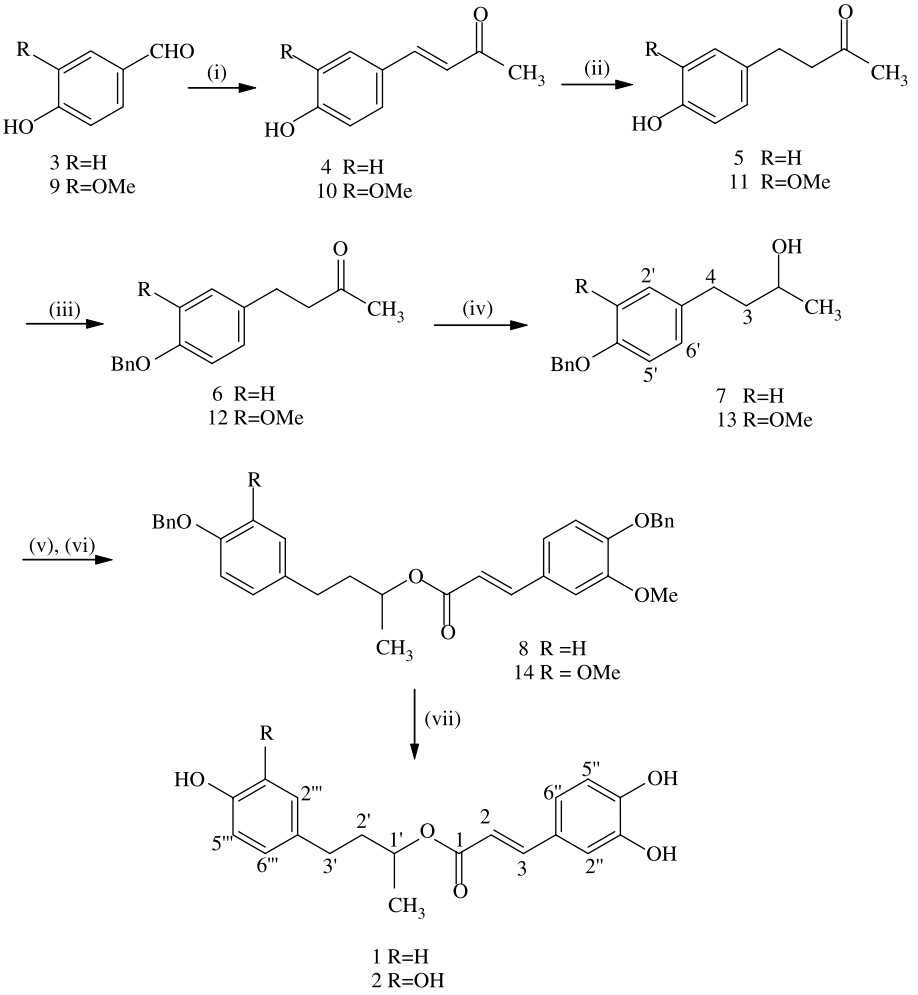
presence of Pd as a catalyst, yielded 4-(4-hydroxyphenyl)-2-butanone (**5**, 89%). The phenolic hydroxyl of **5** was protected as benzyl ether using benzyl bromide in the presence of potassium carbonate [17] to furnish 4-(4-benzyloxyphenyl)-2-butanone (**6**) in 92% yield (scheme 1). Reduction of **6** using sodium borohydride gave 4-(4-benzyloxyphenyl)-2-butanol (**7**) in 90% yield. 4-Benzyloxy-3-methoxycinnamic acid, obtained from 4-benzyloxy-3-methoxybenzaldehyde, was converted into corresponding acid chloride using thionyl chloride. Esterification of **7** with 4-benzyloxy-3-methoxycinnamoyl chloride in presence of 4-dimethylaminopyridine (DMAP) [14] as a catalyst yielded 1′-methyl-3′-(4-benzyloxyphenyl)propyl 4-benzyloxy-3-methoxycinnamate (**8**, 41%). Deprotection of **8** using aluminium chloride [18] gave the title compound 1 (52%, scheme 1). Compound **2** was synthesized by a similar method as described for **1** (scheme 1). $^1$H NMR data of the synthetic **1** and **2** are in good agreement with those reported for the natural products [13]. However, synthetic **1** and **2** are obtained as optically inactive DL-isomeric mixtures, whereas the






Scheme 1. Reagents and conditions: (i) NaOH, acetone, room temperature (rt), 12 h, 4, 83%, 10, 90%; (ii) $H_2$, Pd/C, ethyl acetate, rt, 4 h, 5, 11, 89%; (iii) BnBr, $K_2CO_3$, acetone, reflux, 4 h, 6, 92%, 12, 98%; (iv) $NaBH_4$, methanol, rt, 2 h, 7, 90%, 13, 95%; (v) 4-benzoyl-3-methoxycinnamic acid, $SOCl_2$; (vi) triethylamine, MDC, DMAP, rt, 12 h, 8, 41%, 14, 45%; (vii) $AlCl_3$, DMA, MDC, rt, 12 h, 1, 52%, 2, 40%.

naturally occurring compounds **1** and **2** are *levo* isomers (**1**, $[\alpha]_D$: -27.0° (*c* 0.39, MeOH) and **2**, $[\alpha]_D$: -3.65° (*c* 0.25, MeOH)] [13]. The detailed experimental procedures are described in the section 3.

Synthetic compounds **1** and **2** (DL-isomers) have been evaluated for their anti-oxidative [19] and 5-lipoxegenase inhibitory activities [20]. **1** and **2** exhibited potent anti-oxidative activity in comparison with the commercially available antioxidants, butylated hydroxytoluine (BHT), butylated hydroxyanisole (BHA), vitamin C and vitamin A, in both the nitroblue tetrazolium (NBT) (BHT, $IC_{50}$: 381; BHA, $IC_{50}$: 966; vitamin C, $IC_{50}$: 852; vitamin A, $IC_{50}$: $>$ 1000; 1, $IC_{50}$: 52 and 2, $IC_{50}$: 14 μM) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-models (BHT, $IC_{50}$: 19; BHA, $IC_{50}$: 18; vitamin C, $IC_{50}$: 14; vitamin-A, $IC_{50}$: $>$ 1000; **1**, $IC_{50}$: **9** and **2**, $IC_{50}$: 8 μM), respectively. In addition, compound **1** also showed moderate 5-lipoxegenase inhibitory activity (56%), but, **2** did not exhibit any significant 5-lipoxegenase inhibitory activity.



## 3. Experimental

### *3.1 General experimental procedures*

Melting points were recorded on a V Scientific melting-point apparatus, in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer BX1 FTIR spectrophotometer; $^1$H NMR spectra were recorded on Varian Gemini 200, 300 and 400 MHz NMR spectrometers; the values for chemical shifts ($\delta$) are given in ppm and coupling constants (*J*) in hertz (Hz). Mass spectra were recorded on an Agilent 1100 LC/MSD instrument. 4-Benzyloxy-3-methoxybenzaldehyde [17] and 4-benzyloxy-3-methoxycinnamic acid [21] were prepared according to the procedures described in the literature.

### *3.2 Synthesis of compounds*

**3.2.1 4-(4-Hydroxyphenyl)-3-buten-2-one (4)**. To the solution of 4-hydroxybenzaldehyde (**3**, 10.0 g, 81.96 mmol) in acetone (60 ml) was added aq. sodium hydroxide solution (20%, 50 ml) and this was stirred overnight at room temperature. The reaction mixture was diluted with ice-cold water and acidified with conc. HCl (70 ml). The brown precipitate formed was filtered, washed with cold water and dried to obtain **4** (11.0 g, 83%), mp 100-102°C (lit. [22], mp 95-97°C).

**3.2.2 4-(4-Hydroxy-3-methoxyphenyl)-3-buten-2-one (10)**. 10 was prepared from vanillin (9, 10.0 g, 65.78 mmol) by following the same procedure as described for **4** (11.36 g, 90%), brown solid, mp 126-128°C (lit. [23], mp 128-129°C).

**3.2.3 4-(4-Hydroxyphenyl)-2-butanone (5)**. To a stirred solution of **4** (5.0 g, 30.86 mmol) in ethyl acetate (100 ml) was added palladium (10% on carbon, 100 mg) and this was stirred for 4 h under hydrogen atmosphere at room temperature. The reaction mixture was filtered, washed with ethyl acetate and the crude product obtained by the evaporation of the solvent

was chromatographed using hexane/ethyl acetate (75:25) as eluent to obtain 5 (4.5 g, 89%), colourless solid, mp 74-76°C (lit. [22], mp 75-76°C).

**3.2.4 4-(4-Hydroxy-3-methoxyphenyl)-2-butanone (11)**. **11** was prepared by the hydrogenation of **10** (5.0 g) by adopting the same procedure as described for the preparation of **5** (4.51 g, 89%), low melting solid (lit. [23], mp 40-41°C).

**3.2.5 4-(4-Benzyloxyphenyl)-2-butanone (6) [24]**. A mixture of **5** (9.14 g, 55.73 mmol), potassium carbonate (19.22 g, 139.32 mmol) and benzyl bromide (7.95 ml, 66.87 mmol) in acetone (160 ml) was refluxed for 4 h. The inorganic salt formed was filtered, washed with acetone and crude product was purified further by column chromatography on a silica gel column using hexane/ethyl acetate (80:20) as eluent to furnish **6** (12.75 g, 92%), colourless solid, mp 80-82°C.



**3.2.6 4-(4-Benzyloxy-3-methoxyphenyl)-2-butanone (12) [25]**. 4-(4-Benzyloxy-3-methoxyphenyl)-2-butanone (**12**) was prepared by adopting the procedure of synthesis of **6**, starting from 11 (8.9 g) as an oil (12.75 g, 98%).

**3.2.7 4-(4-Benzyloxyphenyl)-2-butanol (7)**. To a stirred solution of **6** (12.96 g, 51.02 mmol) in methanol (100 ml) was added sodium borohydride (2.83 g, 74.47 mmol) in portions at 10°C over a period of 30 min. After 2 h stirring at room temperature and on completion of the reaction, methanol was removed under reduced pressure and the crude product was diluted with ice-cold water. The solid formed after acidification with dil. HCl (20%, 50 ml) was filtered, washed with cold water and dried to obtain 7 (11.75 g, 90%), colourless solid, mp 61-63°C; IR (neat) $\nu_{max}$ 3374, 2919, 1608, 1511, 1450, 1247, 810, 738 $cm^{-1}$; NMR $\delta_H$ (300 MHz, $CDCl_3$): 1.25 (3H, d, $J = 6.5$ Hz, H-1), 1.75 (2H, m, H-3), 2.69 (2H, m, H-4), 3.70 (1H, m, H-2), 5.05 (2H, s, H-$ArOCH_2$-), 6.85 (2H, d, $J = 8.0$ Hz, H-3′, 5′), 7.11 (2H, d, $J = 8.0$ Hz, H-2′, 6′), 7.26-7.60 (5H, m, H-$ArOCH_2C_6H_5$); LCMS (ESI, positive scan) *m/z* 279 $[M + Na]^+$.

**3.2.8 4-(4-Benzyloxy-3-methoxyphenyl)-2-butanol (13)**. By following the same procedure as described for the preparation of **7**, **13** was obtained from 12 (12.0 g) (11.5 g, 95%), colourless solid, mp 54-56°C; IR (neat) $\nu_{max}$ 3392, 2930, 1589, 1454, 1139, 1032, 737 $cm^{-1}$; LCMS (ESI, positive scan) *m/z* 309 $[M + Na]^+$.

**3.2.9 1′-Methyl-3′-(4-benzyloxyphenyl)propyl 4-benzyloxy-3-methoxycinnamate (8)**. To a stirred mixture of **7** (1.79 g, 6.99 mmol), triethylamine (4 ml, 28.16 mmol) and DMAP (50 mg) in methylenedichloride (MDC) (20 ml) was added 4-benzyloxy-3-methoxycinnamoyl chloride [prepared from corresponding acid (2.0 g) and thionyl chloride (4 ml)] in MDC (25 ml) drop-wise at room temperature for 15 min. After stirring overnight at room temperature, the reaction mixture was poured into ice-cold water (50 ml) and added to dil. HCl (20%, 50 ml). On extraction with chloroform (4 × 50 ml), the combined chloroform layer was washed with water and brine and finally dried over

anhydrous $Na_2SO_4$. The crude product obtained by the evaporation of the solvent was purified by column chromatography on a silica gel column using hexane/ethyl acetate (70:30) as eluent to yield **8** as an oil (1.49 g, 41%); IR (neat) $\nu_{max}$ 2932, 1703, 1632, 1511, 1256, 1023, 738 $cm^{-1}$; NMR $\delta_H$ (300 MHz, $CDCl_3$): 1.40 (3H, d, $J = 6.5$ Hz, H-$CH_3$), 1.81 and 1.96 (2H, m, H-2′), 2.62 (2H, m, H-3′), 3.92 (3H, s, H-Ar$OCH_3$), 4.92 (1H, m, H-1′), 5.25 (4H, s, H-2 × Ar$OCH_2$-), 6.23 (1H, d, $J = 16.0$ Hz, H-2), 6.80 (4H, m, H-5″, 2″, 3″, 5″), 7.05 (3H, m, H-2″, 6″, 6″), 7.26-7.40 (10H, m, H-2 × Ar$OCH_2C_6H_5$), 7.51 (1H, d, $J = 16.0$ Hz, H-3); LCMS (ESI, positive scan) *m/z* 545 $[M + Na]^+$.

**3.2.10 1′-Methyl-3′-(4-benzyloxy-3-methoxyphenyl)propyl 4-benzyloxy-3-methoxycinnamate (14)**. By adopting the same experimental procedure as described for the synthesis of **8**, 1′-methyl-3′-(4-benzyloxy-3-methoxyphenyl)propyl 4-benzyloxy-3-methoxycinnamate (**14**) was prepared starting from **13** (3.0 g, 10.56 mmol) and 4-benzyloxy-3-methoxycinnamoyl chloride [prepared from corresponding acid (3.0 g) and thionyl chloride (6 ml)] as oil (2.6 g, 45%); IR (neat) $\nu_{max}$ 2932, 1701, 1511, 1457, 1259, 1028, 850 $cm^{-1}$; NMR $\delta_H$ (200 MHz, $CDCl_3$): 1.30 (3H, d, $J = 6.5$ Hz, H-$CH_3$), 1.99 (2H, m, H-2′), 2.61 (2H, m, H-3′), 3.89 (3H, s, H-Ar$OCH_3$), 3.92 (3H, s, H-Ar$OCH_3$), 5.01 (1H, m, H-1′), 5.29 (4H, s, H-2 × Ar$OCH_2$-), 6.25 (1H, d, $J = 16.0$ Hz, H-2), 6.63-6.85 (6H, m, H-2″, 5″, 6″, 2″, 5″, 6″), 7.29-7.40 (10H, m, H-2 × Ar$OCH_2C_6H_5$), 7.51 (1H, d, $J = 16.0$ Hz, H-3); LCMS (ESI, positive scan) *m/z* 575 $[M + Na]^+$.

**3.2.11 1′-Methyl-3′-(4-hydroxyphenyl)propyl caffeate (1)**. To a stirred solution of **8** (1.0 g, 1.92 mmol) in MDC (25 ml) was added *N*,*N*-dimethylaniline (DMA) (4.86 ml, 38.4 mmol) and the reaction mixture was cooled to 0°C, followed by the addition of aluminium chloride (4.0 g, 30.07 mmol) at the same temperature. The temperature of the reaction mixture was allowed to come to room temperature and then stirred for overnight at room temperature. It was poured into cold dil. HCl (20%, 50 ml) solution and was extracted with ethyl acetate (5 × 50 ml). The combined ethyl acetate layer was washed with water and brine and dried over anhydrous $Na_2SO_4$. The crude product obtained by the evaporation of the solvent was chromatographed on a silica gel column using hexane/ethyl acetate (60:40) as eluent to yield **1** as light brown oil (330 mg, 52%); IR (neat) $\nu_{max}$ 3338, 1674, 1601, 1516, 1446, 1189, 1054, 816 $cm^{-1}$; NMR $\delta_H$ (400 MHz, methanol-$d_4$): 1.29 (3H, d, $J = 6.2$ Hz, H-$CH_3$), 1.83 and 1.96 (2H, m, H-2′), 2.56 (2H, m, H-3′), 4.90 (1H, m, H-1′, merged with the solvent signal), 6.24 (1H, d, $J = 16.0$ Hz, H-2), 6.71 (2H, d, $J = 8.8$ Hz, H-3″, 5″), 6.78 (1H, d, $J = 8.0$ Hz, H-5″), 6.93 (1H, dd, $J = 8.0$, 2.0 Hz, H-6″), 6.97 (2H, d, $J = 8.8$ Hz, H-2″, 6″), 7.07 (1H, d, $J = 2.0$ Hz, H-2″), 7.54 (1H, d, $J = 16.0$ Hz, H-3); LCMS (ESI, negative scan) *m/z* 327 $[M - H)^-$.

**3.2.12 1′-Methyl-3′-(3,4-dihydroxyphenyl)propyl caffeate (2)**. By adopting the same procedure as described for the synthesis of **1**, 1′-methyl-3′-(3,4-dihydroxyphenyl)propyl caffeate (**2**) was obtained from **14** (890 mg, 1.61 mmol) and aluminium chloride (5.36 g, 40.30 mmol) as light brown oil (221 mg, 40%); IR (neat) $\nu_{max}$ 3336, 1678, 1603, 1518, 1445, 1281, 813 $cm^{-1}$; NMR $\delta_H$ (400 MHz, methanol-$d_4$): 1.30 (3H, d, $J = 6.5$ Hz, H-$CH_3$), 1.82 and 1.93 (2H, m, H-2′), 2.49 (2H, m, H-3′), 4.90 (1H, m, H-1′, merged with the solvent signal), 6.23 (1H, d, $J = 16.0$ Hz, H-2), 6.44 (1H, dd, $J = 8.0$, 2.0 Hz, H-6″), 6.59 (1H, d,

$J = 2.0$ Hz, H-2″), 6.63 (1H, d, $J = 8.0$ Hz, H-5″), 6.77 (1H, d, $J = 8.0$ Hz, H-5″), 6.93 (1H, dd, $J = 8.0$, 1.6 Hz, H-6″), 7.03 (1H, s, H-2″), 7.52 (1H, d, $J = 16.0$ Hz, H-3); LCMS (ESI, negative scan) *m/z* 343 [M - H]$^-$.

## 4. Bioactivity studies

The anti-oxidative activity of the esters **1** and **2** in both the NBT and DPPH free radical-scavenging mechanisms was determined according to the procedure described in our previous communication [19] and 5-lipoxygenaes inhibitory activity was determined according to the procedure described in the literature [20].



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