# Macrophage Activating Effects of New Alkamides from the Roots of *Echinacea* Species

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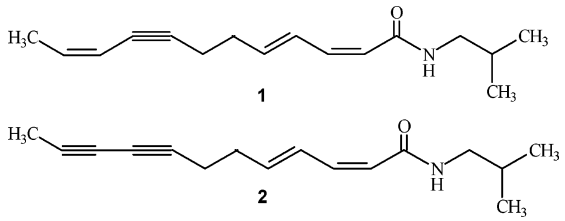
Chemical investigation of the roots of *Echinacea angustifolia, E. purpurea*, and *E. pallida* yielded two new alkamides, identified by analysis of spectroscopic data and comparison with reported alkamides. The new compounds were dodeca-2*Z*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide (**1**) from *E. angustifolia* and dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2**) from *E. purpurea* and *E. pallida*. These two components, as well as previously identified alkamides, exerted inhibition on LPS-mediated activation of a murine macrophage line, RAW264.7. These data suggest that these alkamides may have anti-inflammatory activity. The cytotoxicity of these alkamides using MTT assays was also investigated.

*Echinacea* is one of the best selling herbal medicines in the United States and Europe.[1,2] Of the nine species, *E. angustifolia* DC., *E. pallida* Nutt., and *E. purpurea* (L.) Moench are the most commonly used materials in herbal remedies. Alkamides, caffeic acid derivatives, and polysaccharides are regarded as active constituents that contribute to the immunomodulatory activity and the prevention and treatment of upper respiratory tract infection.[3] Chemical investigation of lipophilic alkamides from the roots *of E. angustifolia*, *E. purpurea*, and *E. pallida* afforded 14 alkamides. Twelve were previously identified as undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide, undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*E*,10*E*-trien-8-ynoic acid isobutylamide, trideca-2*E*,7*Z*-diene-10,12-diynoic acid isobutylamide, dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide, dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide, dodeca-2*E*,4*E*-dienoic acid isobutylamide, undeca-2*Z*-ene-8,10-diynoic acid isobutylamide, and dodeca-2*E*,4*Z*,-10*Z*-trien-8-ynoic acid isobutylamide.[4−6] The remaining two, dodeca-2*Z*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide (**1**) from *E. angustifolia* and dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2**) from *E. purpurea* and *E. pallida*, were new compounds. The anti-inflammatory activity of the two new alkamides, together with eight known compounds, and cytotoxicity of these using MTT assays are also reported here.

EtOH extracts of *E. angustifolia* root, *E. purpurea* root, and *E. pallida* root were condensed under reduced pressure and low temperature and fractionated by solvent partitioning. Silica gel column chromatography followed by further purification using reversed-phase chromatography (RP-18) on preparative HPLC or open column chromatography led to the isolation of 14 alkamides. The identification was conducted by analysis of UV−vis, $^1$H NMR, $^{13}$C NMR, and LCMS spectroscopic data and comparison with reported alkamides, of which two (**1** and **2**) were found to be new.




The presence of an isobutylamide moiety in compounds **1** and **2** was suggested by $[M - 73]^+$, $[M - 99]^+$, and $[M - 101]^+$ ions observed in LCMS spectra of both compounds, which are due to the loss of $H_2NCH_2CH(CH_3)_2$, $O{=}C{=}NCH_2CH(CH_3)_2$, and $O{=}CHNHCH_2CH(CH_3)_2$, respectively.

Characteristic signals at $\delta$ 3.14 (t, H-1′), 1.80 (m, H-2′), and 0.93 (d, H-3′ and H-4′) in the $^1$H NMR spectrum of compound **1** further supported an isobutylamide moiety. Signals of olefinic protons at 5.50 (d, H-2), 6.39 (t, H-3), 7.53 (dd, H-4), and 6.04 (dt, H-5) confirmed the presence of two double bonds conjugated with the amide carbonyl. The *Z*/*E*-stereochemistry of the double bonds was determined by analysis of the coupling constants. A $\gamma$-proton in compound **1** shifted downfield to $\delta$ 7.53 also suggested the *Z*-configuration of the α, $\beta$-double bond due to the effect of anisotropic electron regions of the carbonyl toward the α,$\beta$-*Z*-double bond, whereas a $\beta$-proton should shift furthest downfield in those with *E*-configuration of the α,$\beta$-double bond. The signals for an olefinic proton at 5.45 (dq, H-10) and 5.88 (dq, H-11) and the remaining methyl protons at 1.84 (dd, H-12) were identical to those in dodeca-2*E*,4*Z*,-10*Z*-trien-8-ynoic acid isobutylamide.[6]

The $^1$H NMR of compound **2** was similar to that of undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide,[5] except that compound **2** exhibited a triplet ($J$ = 1.0 Hz) at $\delta$ 1.90 for three protons, while undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide gave a singlet at $\delta$ 1.98 for one proton. This suggested a terminal methyl group instead of a proton attached to the diynoic group in compound **2**, which was further confirmed by the molecular ion at *m*/*z* 243 for **2** and *m*/*z* 229 for undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide in LCMS.

The biological activity of the two new alkamides (**1** and **2**) and undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide, dodeca-

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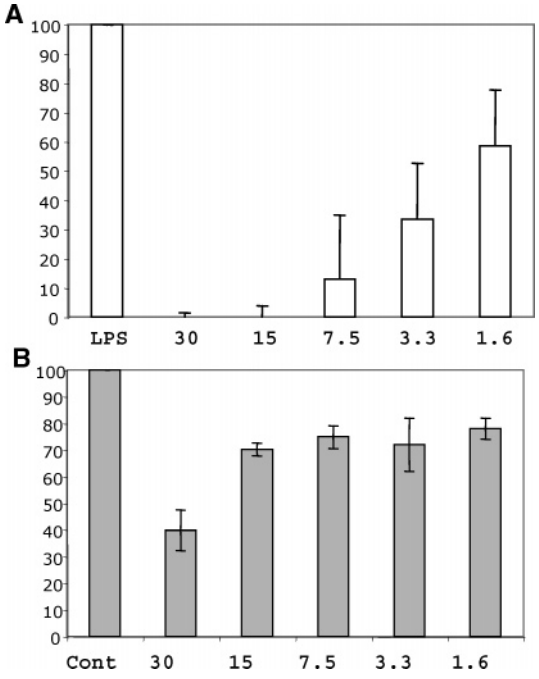


**Figure 1.** Effect of total-alkamides on nitric oxide (NO) production and cell viability. (A) Relative NO production. RAW264.7 cells were incubated with various concentrations of total alkamides (ranging from 1.6 to 30 $\mu$g/mL) for 4 h and followed by LPS stimulation (5 ng/mL) for 24 h. The supernatants were analyzed for NO production. (B) Cell viability. The toxicity of total alkamides was determined using the MTT assay, in which the same dose range of alkamides was used as described in A. Data are expressed as the mean ± standard errors of three independent experiments.

2*E*,4*Z*-diene-8,10-diynoic acid 2-methybutylamide, dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide, dodeca-2*E*,4*E*-dienoic acid isobutylamide, undeca-2*Z*-ene-8,10-diynoic acid isobutylamide, and dodeca-2*E*,4*Z*,10*Z*-trien-8-ynoic acid isobutylamide was evaluated using a murine macrophage cell line, RAW 264.7. This cell line is a well-established model system to study macrophage activation and to assess pro-inflammatory or anti-inflammatory activity of various compounds and extracts.[7,8] To establish a robust screening procedure, the Griess assay was adopted to assess NO production, which parallels the pro-inflammatory activation. Lipopolysaccharide (LPS), a known macrophage activator, was included as our positive control. Initially, we attempted to determine whether any of the alkamides could activate RAW 264.7, but failed to demonstrate that. On the other hand, when the cells were preincubated with total-alkamides (i.e., including all alkamide components in their appropriate ratio) then stimulated with LPS, there was a significant reduction in NO production as compared to the cells treated with LPS only (Figure 1A). To determine whether the observed inhibition in NO production could be attributed to nonspecific cytotoxicity of total-alkamides, since these compounds are highly lipophilic, we performed cell viability tests on the same cultured cells by using the MTT assay (Molecular Probe). As shown in Figure 1B, total-alkamides showed significant inhibition in cell viability only at 30 $\mu$g/mL. On the other hand, much lower concentration was required to suppress NO production (Figure 1A). The reduction was observed even in the culture preincubated with alkamides for only 4 h, and the cells were washed before stimulation with LPS. The cell viability and NO production of these treated cells appeared comparable to the ones with the compounds present throughout the incubation period (i.e., 24 h, unpublished observation). This finding indicates that the effect caused by alkamides occurs rather promptly and appears to be irreversible.

We extended our analyses to the individual alkamide components, including newly isolated ones, in which the effect on cell viability and LPS-mediated NO production were compared side by side. A dose-dependent study was performed to determine the inhibition dose that causes 50% reduction in comparison to LPS control, i.e., $ID_{50}$, as well as toxicity doses that result in 50% cell death (i.e., $TD_{50}$). As summarized in Table 1, $ID_{50}$ for many of the alkamides was around 2−5 $\mu$g/mL. On the other hand, their toxicity dose ranges were comparable, and around 25−50 $\mu$g/mL. Thus, the concentration required to suppress NO production is lower than that which induces cell death. These data indicate that alkamides can suppress LPS-mediated macrophage activation without causing significant cell death. Thus, these compounds can potentially have anti-inflammatory activity. However, they may still be toxic to the cells treated at low concentrations (Figure 1B). In addition, this inhibitory effect proceeds rather quickly within 4 h incubation with alkamides and becomes nonreversible after removing the compounds. It remains to be determined what targets alkamides interact with and how the interaction blocks LPS-induced NO production.

## Experimental Section

**General Experimental Procedures.** UV spectra were recorded on a Buck Scientific CECIL CE2021 UV−vis spectrophotometer. NMR spectra were measured on a Bruker Advance-400 spectrometer. HRMS were recorded on a Finnigan MAT-95 spectrometer. LCMS was run on a Hitachi model M-8000 LC/3DQ system (SSI ion source, Hitachi L-2100 pump, and L-2400 UV detector). Preparative HPLC was run on a Varian PrepStar (ProStar SD-1 pumps and ProStar 330 photodiode array detector). LCMS was run with a gradient (50% MeCN increase to 70% in 7 min, then to 90% in 7 min, and then increase to 100% in 6 min), flow rate 0.8 mL/min, 260 nm UV detector, 1/4 split to MS (positive ion polarity, accumulation voltage 0 V, cover plate 150 °C, aperture 1 100

**Table 1.** Inhibition of LPS-Mediated Activation and Toxicity of Alkamides

| compound | $ID_{50}$ ($\mu$g/mL) | $TD_{50}$ ($\mu$g/mL) |
|---|---|---|
| undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide | 12.5 | 50 |
| dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide | 3 | 26 |
| dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide | 3 | 25 |
| dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide | 6 | 50 |
| dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide | 6 | 50 |
| dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide | 10 | 24 |
| dodeca-2*E*,4*E*-dienoic acid isobutylamide | 6 | 12.5 |
| undeca-2*Z*-ene-8,10-diynoic acid isobutylamide | 25 | 50 |
| dodeca-2*E*,4*Z*,10*Z*-trien-8-ynoic acid isobutylamide | 6 | 40 |
| dodeca-2*Z*, 4*E*, 10*Z*-trien-8-ynoic acid isobutylamide (**1**) | 12 | 30 |
| dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2**) | 8 | 25 |
| total alkamides | 6 | 12.5 |

°C, aperture 2 120 °C, detector 450 °C, focus 30 V, drift 40 V, SSI chamber 0 KV, ASC and centroiding off).

**Plant Material.** The dried roots of *Echinacea angustifolia* (EAR-W1020-W) were purchased from Trout Lake Farm, Trout Lake, WA. The roots of *Echinacea purpurea* (EPR110902F) were collected from Gaia Farm, Brevard, NC (seeds were purchased from Johnny's Selected Seeds, Albion, ME). To confirm this species, chromosome number and chromosome karyotypes were analyzed.[9] The dried roots of *Echinacea pallida* (WA8141) were purchased from Strategic Sourcing, Banner Elk, NC. All materials were identified morphologically with microscopy. DNA fingerprint comparison with the authentic species was conducted in this Lab. The voucher specimens of *E. purpurea* and the reference samples of *E. angustifolia* and *E. pallida* were kept at Gaia Herbs, Inc.

**Extraction and Isolation.** *E. angustifolia* (10 kg) roots were powdered and extracted with 80% EtOH at room temperature. After concentration of the combined extracts under reduced pressure at 40 °C, the residue was partitioned between hexane and 90% MeOH. The hexane layer (84 g) was subsequently chromatographed on a silica gel column (700 g, 60 × 7.5 cm, 230−450 mesh) and eluted with a 98:2, 95:5, 90:10, 85:15, and 80:20 hexane/$CHCl_3$ mixture. Fractions of 500 mL were collected, concentrated, and chromatographed on TLC plates (Merck TLC plates RP-18 F254 0.25 mm). The fractions showing spots on TLC plates with similar $R_f$ values under 254 nm were combined. From the 20% $CHCl_3$ fraction, 25 mg of dodeca-2*Z*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide (**1**) was finally obtained using preparative HPLC (Miorosorb RP-18, Varian Dynamax 250 × 41.4 mm) with gradient MeCN−$H_2O$ as eluent. Seven known compounds, undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide/dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide, dodeca-2*E*,4*E*-dienoic aicd isobutylamide, undeca-2*Z*-ene-8,10-diynoic acid isobutylamide, and dodeca-2*E*,4*Z*,10*Z*-trien-8-ynoic acid isobutylamide, were additionally purified.

*E. purpurea* roots (5 kg) were ground and extracted with 70% EtOH. The extract was condensed and partitioned between hexane and 90% MeOH. The hexane layer (60 g) was combined and chromatographed on a silica gel column (700 g, 60 × 7.5 cm, 230−450 mesh) and eluted with a 95:5, 90:10, 85:15, and 80:20 hexane/$CHCl_3$ mixture. From the 20% $CHCl_3$ fraction, 420 mg of dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2**) was purified by open RP-18 column (3 × 34 cm) eluted by MeOH/MeCN/$H_2O$ (1:1:3). Further, nine known alkamides, undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide, undeca-2*Z*,4*E*-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*E*,-10*E*-trien-8-ynoic acid isobutylamide, trideca-2*E*,7*Z*-diene-10,12-diynoic acid isobutylamide, dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide/dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide, and dodeca-2*E*,4*Z*,10*Z*-trien-8-ynoic acid isobutylamide, were also obtained.

*E. pallida* roots (10 kg) was ground and extracted with 70% EtOH and partitioned between $CHCl_3$ and $H_2O$. The $CHCl_3$ layer was then partitioned between 90% MeOH and hexane. Hexane and MeOH layers were concentrated to give 20 and 4 g, respectively. The methanol-soluble portion (4 g) was mixed with 5 g of silica and then placed on a silica gel column (120 g, 22 × 4 cm, 230−450 mesh), eluted with 90:10, 85:15, and 80:20 hexane/$CHCl_3$ mixtures. Fractions of 200 mL each were collected and concentrated. Fractions containing similar spots on TLC plates were combined and chromatographed further on an open RP-18 column (3 × 34 cm) eluted by MeOH/MeCN/$H_2O$ (1:1:3). Finally, 56 mg of dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2**) together with undeca-2*Z*,4*E*-8,10-diynoic acid isobutylamide, tetradeca-8*Z*-ene-11,13-diyn-2-one, and pentadeca-8*Z*,11*Z*,13*E*-trien-2-one, and pentadeca-8*Z*,-11*E*,13*Z*-trien-2-one were obtained.

**Dodeca-2*Z*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide (1):** colorless viscous oil; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 257 (4.10); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.93 (6H, d, $J$ = 6.6 Hz, $H_3$-3′ and $H_3$-4′), 1.80 (1H, m, H-2′), 1.84 (3H, dd, $J$ = 1.8, 6.8 Hz, $H_3$-12), 2.45 (4H, m, $H_2$-6 and $H_2$-7), 3.14 (2H, t, $J$ = 6.6 Hz, $H_2$-1′), 5.45 (1H, dq, $J$ = 10.6, 1.8 Hz, H-10), 5.50 (1H, d, $J$ = 11 Hz, H-2), 5.52 (1H, brs, N−H), 5.88 (1H, dq, $J$ = 10.6, 6.8 Hz, H-11), 6.04 (1H, dt, $J$ = 15, 6.4 Hz, H-5), 6.39 (1H, dd, $J$ = 11, 11 Hz, H-3), 7.53 (1H, dd, $J$ = 11, 15 Hz, H-4); $^{13}$C NMR and DEPT ($CDCl_3$, 100 MHz) $\delta$ 166.35 (C, C-1), 141.03 (CH, C-3), 140.96 (CH, C-5), 137.34 (CH, C-11), 127.95 (CH, C-4), 119.26 (CH, C-2), 110.20 (CH, C-10), 93.67 (C, C-8), 77.79 (C, C-9), 46.87 ($CH_2$, C-1′), 32.24 ($CH_2$, C-6), 28.58 ($CH_2$, C-7), 20.16 ($CH_3$, C-3′,4′), 19.15 (CH, C-2′), 15.73 ($CH_3$, C-12); LCMS UV and TIC (total ion chromatography) spectra gave a peak at retention time 10.49; mass spectra extracted at RT 10.49 gave *m/z* 490.9 $[2M]^+$, 246.8 $[M + H]^+$, 245.8 $[M]^+$, 172.6 $[M - H_2NC_4H_9]^+$, 146.7 $[M - OCNC_4H_9]^+$, 144.6 $[M - HCONHC_4H_9]^+$. Inhibition of LPS-mediated activation: $ID_{50}$ 5 $\mu$g/mL. Toxicity: 50 $\mu$g/mL. HRMS (EI): *m/z* 245.1773 $[M]^+$ (calcd for $C_{16}H_{23}NO$ 245.1779).

**Dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (2):** white microcrystal; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 258 (4.44); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.93 (6H, d, $J$ = 6.7 Hz, $H_3$-3′ and $H_3$-4′), 1.82 (1H, m, H-2′), 1.90 (3H, t, $J$ = 1.0 Hz, $H_3$-12), 2.38 (4H, m, $H_2$-6 and $H_2$-7), 3.13 (2H, dd, $J$ = 6.3, 6.7 Hz, $H_2$-1′), 5.52 (1H, d, $J$ = 11 Hz, H-2), 5.53 (1H, brs, N-H), 5.98 (1H, dt, $J$ = 15, 7 Hz, H-5), 6.38 (1H, dd, $J$ = 11, 11 Hz, H-3), 7.48 (1H, ddd, $J$ = 1.0, 11, 15 Hz, H-4); $^{13}$C NMR and DEPT ($CDCl_3$, 100 MHz) $\delta$ 166.31 (C, C-1), 140.80 (CH, C-3), 140.13 (CH, C-5), 128.12 (CH, C-4), 119.56 (CH, C-2), 75.59 (C, C-8), 73.44 (C, C-9), 65.93 (C, C-10), 64.43 (C, C-11), 46.70 ($CH_2$, C-1′), 31.60 ($CH_2$, C-6), 28.59 ($CH_2$, C-7), 20.16 ($CH_3$, C-3′,4′), 19.06 (CH, C-2′), 4.18 ($CH_3$, C-12); LCMS UV and TIC (total ion chromatography) spectra gave peak at retention time 9.35; mass spectra extracted at RT 10.49 gave *m/z* 487.2 $[2M]^+$, 244.8 $[M + H]^+$, 243.9 $[M]^+$, 170.8 $[M - H_2NC_4H_9]^+$, 144.7 $[M - OCNC_4H_9]^+$, 142.6 $[M - HCONHC_4H_9]^+$. Inhibition of LPS-mediated activation: $ID_{50}$ 25 $\mu$g/mL. Toxicity: 50 $\mu$g/mL. HRMS (CI, $CH_4$): *m/z* 244.1704 $[M + H]^+$ (calcd for $C_{16}H_{22}NO$ 244.1701).

**Bioassay.** Raw 264.7, a murine macrophage cell line, was preincubated with various concentrations of alkamides for 4 h and then stimulated with 5 ng/mL LPS for 24 h. In some experiments, after the preincubation with alkamides, the culture medium containing these compounds was removed and cells were washed several times before subjected to LPS stimulation. The supernatants of these cultures were taken for analyses of nitrite production, an indicator of NO synthesis. Specifically, the supernatants were mixed with Griess reagents (Promega), and their absorbance at 550 nm ($A_{550}$) was measured using VersaMax (Molecular Devices). The relative NO production was derived by (Exp-OD − Control-OD)/(LPS-OD − Control-OD) × 100%.

MTT (Molecular Probe) assays were used to determine the cytotoxicity of alkamides following the manufacture's instruction. Briefly, cells in 96-well plates were incubated with various concentrations of alkamides for 24 h (or 4 h followed by 24 h in fresh medium). The cells were then washed, labeled with MTT reagents for 4 h, and lysed with DMSO for the determination of formazan conversion using VersaMax (Molecular Devices) at absorbance $A_{540}$. The relative cell viability was determined by a percentage of experimental OD to the control OD that had the same cell number as the experimental group, i.e., Exp-OD/Control-OD × 100%.

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## References and Notes

(1) Brevoort, P. *HerbalGram* **1998**, 33−48.
(2) Bauer, R. In *Phytomedicines of Europe. Chemistry and Biological Activity*; Lawson, L. D., Bauer, R., Eds.; American Chemical Society: Washington, DC, 1998; pp 140−157.

(3) Bauer, R.; Wagner, H. In *Economic and Medicinal Plant Research*; Wagner, H., Farnsworth, N. R., Eds.; Academic Press: New York, 1991; Vol. 5, pp 254−321.
(4) Bauer, R.; Remiger, P. *Planta Med.* **1989**, *55*, 367−371.
(5) Bauer, R.; Remiger, P.; Wagner, H. *Phytochemistry* **1988**, *27*, 2339−2342.
(6) Bauer, R.; Remiger, P.; Wagner, H. *Phytochemistry* **1989**, *28*, 505−508.
(7) Murakami, A.; Takahashi, D.; Hagihara, K.; Koshimizu, K.; Ohigashi, H. *Biosci. Biotechnol. Biochem*. **2003**, *67*, 1056−1062.
(8) Shin, N. R.; Lee, D. Y.; Shin, S. J.; Kim, K. S.; Yoo, H. S. *FEMS Immunol. Med. Microbiol*. **2004**, *41*, 169−176.
(9) Qu, L.; Wang, X.; Hood, E.; Wang, M.; Scalzo, R. *HortScience* **2004**, *39*, 368−370.

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