# Supplementary Information

## *Boesenbergia rotunda* and its pinostrobin for atopic dermatitis: Dual 5-lipoxygenase and cyclooxygenase-2 inhibitor and its mechanistic study through steady-state kinetics and molecular modeling

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## 1. Extraction yield (%) of essential oils and hexane extracts derived from the selected medicinal plants

**Table S1.** List of selected medicinal plants and percentage yields of their nonpolar fractions (essential oils and hexane extracts).

| No. | Plant species | Part used | Essential oils | | Hexane extracts | |
|---|---|---|---|---|---|---|
| | | | Yield (%) | Characteristics | Yield (%) | Characteristics |
| 1 | *A. vulgaris* L. | Aerial part | 0.183 | Yellow clear liquid | 4.01 | Crude extract |
| 2 | *B. rotunda* (L.) Mansf. | Rhizome | 0.216 | Yellowish clear liquid | 2.43 | Oily crude extract |
| 3 | *C. sinensis* (L.) Kuntze | Young leaves | 0.016 | Yellowish waxy liquid | 5.74 | Crude extract |
| 4 | *C. fistula* L. | Leaves | - | - | 2.37 | Crude extract |
| | | Pods | - | - | 0.63 | Crude extract |
| 5 | *D. longan* Lour. | Peel | 0.013 | Yellowish liquid | 0.44 | Crude extract |
| | | Seed | - | - | 2.19 | Fixed oils |
| 6 | *M. villosa* Huds. | Aerial part | 0.012 | Cloudy, waxy greenish liquid | 1.58 | Crude extract |
| 7 | *W. trilobata* (L.) Hitchc. | Leaves | 0.086 | Greenish clear liquid | 2.53 | Crude extract |

## 2. Total phenolic content of all plant extracts

**Table S2.** Determination of total phenolic content (TPC) values of all selected plants.

| No | Sample | TPC value (mg gallic acid/g dry weight of extract) | | | |
|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **Mean ± SD** |
| 1 | UAE BR | 24.567 | 23.280 | 25.158 | $24.335 \pm 0.960^{i}$ |
| 2 | EO MV | 20.184 | 21.610 | 19.628 | $20.474 \pm 1.023^{h}$ |
| 3 | UAE AV | 16.428 | 18.584 | 16.045 | $17.019 \pm 1.369^{gh}$ |
| 4 | UAE CSD | 17.610 | 16.845 | 15.315 | $16.590 \pm 1.169^{g}$ |
| 5 | UAE DLP | 15.697 | 15.419 | 15.489 | $15.535 \pm 0.145^{f}$ |
| 6 | EO DLP | 11.419 | 11.558 | 10.097 | $11.025 \pm 0.806^{e}$ |
| 7 | UAE MV | 9.228 | 8.671 | 7.419 | $8.439 \pm 0.926^{d}$ |
| 8 | EO BR | 6.967 | 6.480 | 6.306 | $6.584 \pm 0.343^{c}$ |
| 9 | UAE WT | 6.306 | 4.984 | 5.193 | $5.494 \pm 0.711^{bc}$ |
| 10 | UAE CFF | 4.184 | 4.010 | 5.019 | $4.405 \pm 0.960^{b}$ |
| 11 | EO WT | 0.650 | 0.758 | 0.758 | $0.722 \pm 0.062^{a}$ |
| 12 | UAE CFL | 0.483 | 0.574 | 0.591 | $0.550 \pm 0.058^{a}$ |
| 13 | UAE DLS | 0.289 | 0.313 | 0.376 | $0.326 \pm 0.045^{a}$ |
| 14 | EO AV | 0.268 | 0.344 | 0.299 | $0.304 \pm 0.038^{a}$ |
| 15 | EO CSD | 0.268 | 0.351 | 0.282 | $0.300 \pm 0.046^{a}$ |

*Note: UAE: hexane extract; EO: essentail oils; BR: *Boesenbergia rotunda*; MV: *Mentha villosa*; AV: *Artemisia vulgaris*; CSD: *Camellia sinensis*; DLP: *Dimocarpus longan* peels; DLS: *Dimocarpus longan* seeds; CFF: *Cassia fistula* fruits; WT: *Wedelia trilobata;* and CFL: *Cassia fistula* leaves. One-way ANOVA was performed to compare the mean of each sample, and different letters indicate the significant difference of the mean.

## 3. Total flavonoid content of all plant extracts

**Table S3.** Determination of total flavonoid content (TFC) values of all selected plants.

| No | Sample | TFC value (mg quercetin/g dry weight of extract) | | | |
|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **Mean ± SD** |
| 1 | UAE CSD | 105.851 | 109.957 | 101.904 | 105.904 ± 4.027[d] |
| 2 | UAE MV | 41.264 | 42.384 | 41.957 | 41.868 ± 0.565[c] |
| 3 | UAE CFL | 38.171 | 38.437 | 35.931 | 37.513 ± 1.377[b] |
| 4 | UAE CFF | 5.744 | 6.171 | 5.317 | 5.744 ± 0.427[a] |
| 5 | UAE AV | 5.531 | 5.477 | 5.317 | 5.442 ± 0.111[a] |
| 6 | UAE WT | 5.157 | 5.584 | 5.264 | 5.335 ± 0.222[a] |
| 7 | UAE BR | 4.304 | 3.824 | 4.304 | 4.144 ± 0.244[a] |
| 8 | UAE DLP | 3.131 | 3.557 | 3.291 | 3.326 ± 0.145[a] |

*Note: UAE: hexane extract; CSD: *C. sinensis*; MV: *M. villosa*; CFL: *C. fistula* leaves; CFF: *C. fistula* fruits; AV: *A. vulgaris*; WT: *W. trilobata*; BR: *B. rotunda*; and DLP: *D. longan* peels. One-way ANOVA was performed to compare the mean of each sample, and different letters indicate the significant difference of the mean.

## 4. Gas chromatography–mass spectrometry of *Boesenbergia rotunda* essential oils and hexane extracts

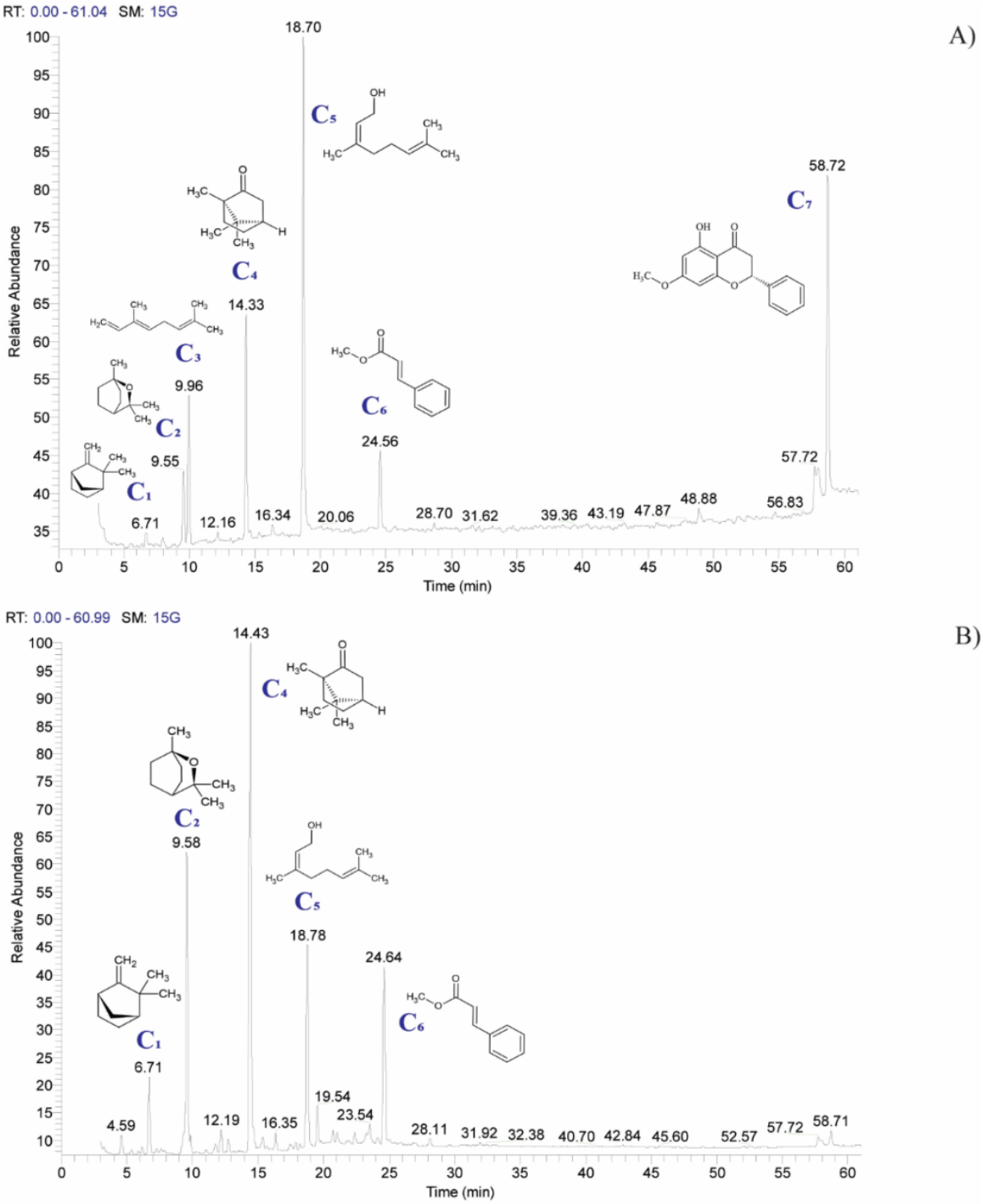



**Figure S1**. Gas Chromatography (GC) chromatograms of A). hexane extract and B). EOs of *B. rotunda*, and their major volatile components (C): camphene ($C_1$), 1.8-cineole ($C_2$), β-cis-ocimene ($C_3$), camphor ($C_4$), nerol ($C_5$), methyl cinnamate ($C_6$), and pinostrobin ($C_7$).

**Table S4.** Chemical composition of volatile compounds of essential oils and hexane extract derived from *B. rotunda*.

| No. | RT* (min) | Compound | % Abundance EOs | Hexane extract |
|---|---|---|---|---|
| 1 | 4.59 | 2-Hexenal | 1.30 | - |
| 2 | 6.71 | Camphene | 4.97 | 1.57 |
| 3 | 9.58 | 1,8-cineole | 20.47 | 4.80 |
| 4 | 9.96 | β-cis-ocimene | - | 8.77 |
| 5 | 12.19 | Linalool | 1.36 | 1.09 |
| 6 | 14.43 | Camphor | 35.25 | 13.99 |
| 7 | 15.36 | Borneol | 1.36 | - |
| 8 | 16.35 | $\alpha$-terpineol | 1.06 | 1.17 |
| 9 | 18.78 | Nerol | 13.86 | 32.76 |
| 10 | 19.54 | Citral | 2.92 | - |
| 11 | 22.38 | cis-Limonene oxide | 0.69 | - |
| 12 | 23.54 | Limonene oxide | 2.36 | - |
| 13 | 24.64 | Methyl cinnamate | 11.71 | 5.46 |
| 14 | 28.7 | (E) $\alpha$-Bergamotene | - | 0.88 |
| 15 | 48.88 | (Z) 6-Pentadecen-1-ol | - | 1.50 |
| 16 | 57.72 | (Z) 9-Octadecenamide | 1.34 | 6.79 |
| 17 | 58.72 | Pinostrobin | 1.33 | 21.22 |

*RT based on the GC chromatogram of essential oils derived from *B. rotunda*.

## 5. Gas chromatography–mass spectrometry of *Camellia sinensis* essential oils

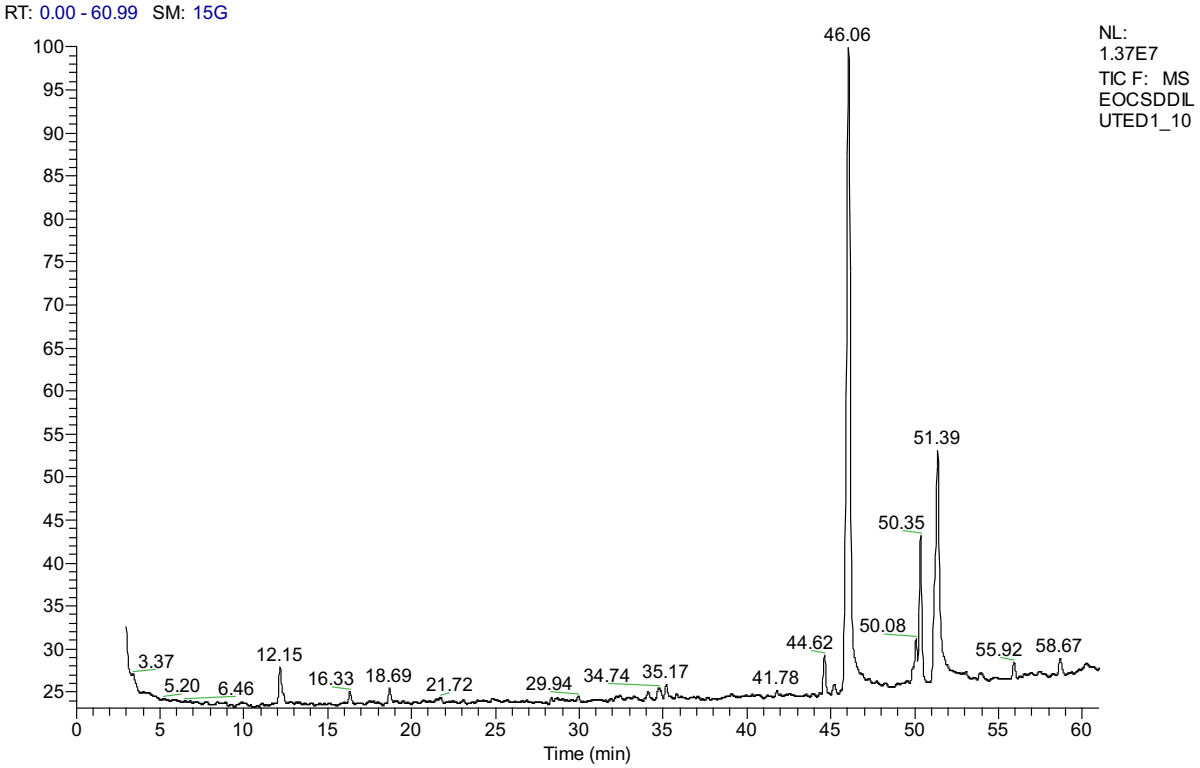



**Figure S2.** GC chromatogram of essential oils derived from *C. sinensis*.

**Table S5.** Chemical composition of volatile compounds in *C. sinensis* essential oils detected by GC–MS analysis.

| No | RT (min) | % Abundance | R Match | Compound |
|---|---|---|---|---|
| 1 | 12.15 | 2.72 | 898 | Linalool |
| 2 | 16.33 | 1.21 | 870 | $\alpha$-terpineol |
| 3 | 18.69 | 1.37 | 829 | Nerol |
| 4 | 20.7 | 0.33 | 787 | 2,4-Decadienal |
| 5 | 21.72 | 0.79 | 835 | Bicyclo [2.1.0] pentane, 1,4-dimethyl- |
| 6 | 23.06 | 0.26 | 900 | Naphthalene, 1,2-dihydro-4,5,7-trimethyl- |
| 7 | 24.82 | 0.35 | 854 | Phenol, 4-(1,1-dimethylpropyl)- |
| 8 | 28.32 | 0.36 | 847 | β-ionone (E) |
| 9 | 29.94 | 0.351 | 824 | Calamenene (Z) |
| 10 | 34.1 | 0.73 | 835 | Cubenol |
| 11 | 34.74 | 1.04 | 895 | Murrolol <epi-alpha-> |
| 12 | 35.17 | 0.98 | 937 | Murrolol <epi-alpha-> |
| 13 | 35.8 | 0.48 | 809 | Cadalene |
| 14 | 44.62 | 2.37 | 904 | Methyl hexadecanoate |
| 15 | 46.06 | 52.54 | 903 | n-hexadecanoic acid |
| 16 | 50.35 | 11.28 | 876 | Phytol |
| 17 | 51.39 | 21.98 | 901 | Linolenic acid, methyl ester |
| 18 | 55.92 | 0.85 | 856 | Tricosane |

## 6. Gas chromatography–mass spectrometry of *Wedelia trilobata* essential oils

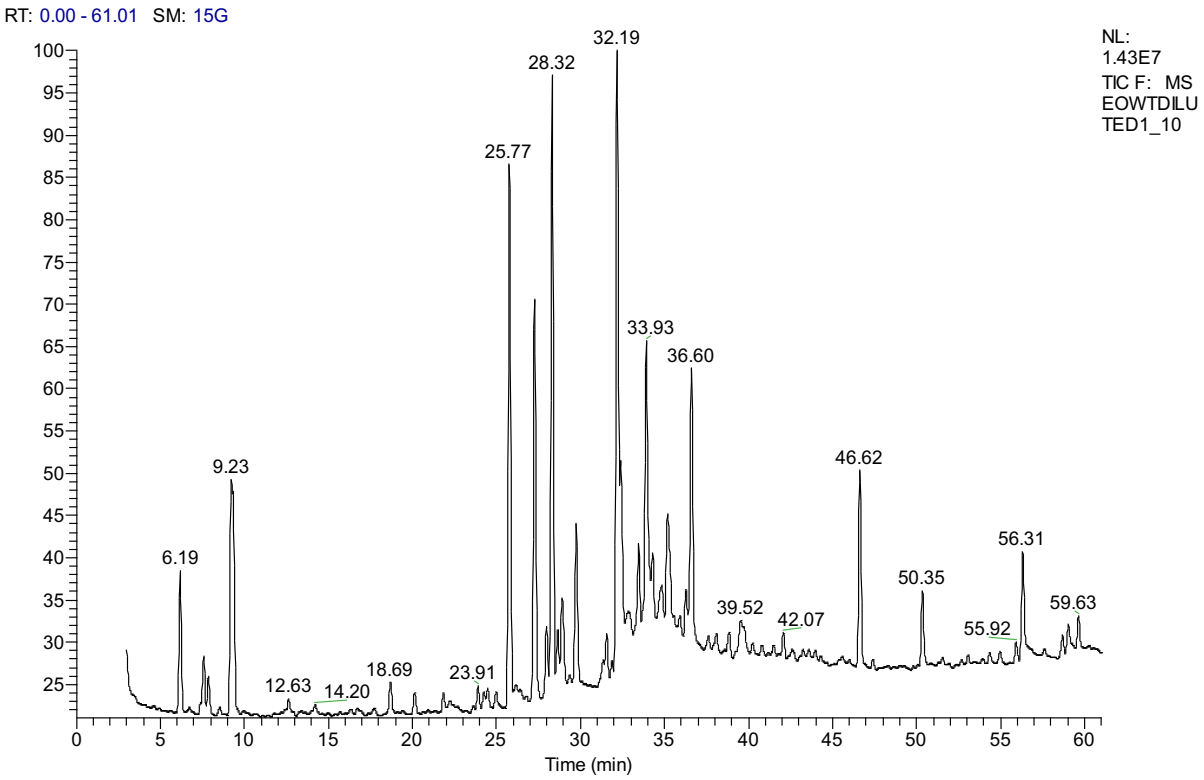



**Figure S3.** GC chromatogram of essential oils derived from *W. trilobata*.

**Table S6.** Chemical composition of volatile compounds in *W. trilobata* essential oils detected by GC–MS analysis.

| No | RT (min) | % Abundance | R Match | Compound |
|---|---|---|---|---|
| 1 | 6.19 | 2.51 | 944 | α-pinene |
| 2 | 6.71 | 0.21 | 887 | Camphene |
| 3 | 7.58 | 1.25 | 940 | (-)-β-Pinene |
| 4 | 8.53 | 0.18 | 861 | α-Phellandrene |
| 5 | 9.23 | 7.50 | 911 | o-Cymene |
| 6 | 12.63 | 0.73 | 798 | Perillene |
| 7 | 18.69 | 0.69 | 865 | Eucarvone |
| 8 | 20.14 | 0.44 | 919 | Bornyl acetate |
| 9 | 21.84 | 0.37 | 788 | Spiro [4.5] decan-6-one |
| 10 | 23.91 | 0.63 | 918 | α-copaene |
| 11 | 24.47 | 0.87 | 916 | α-guaiene |
| 12 | 25.77 | 9.88 | 962 | β-caryophyllene(E-) |
| 13 | 27.27 | 7.63 | 935 | α-humulene |
| 14 | 28.32 | 12.51 | 925 | Germacrene D |
| 15 | 28.9 | 3.09 | 917 | γ-elemene |
| 16 | 29.75 | 3.37 | 916 | δ-cadinene |
| 17 | 31.55 | 1.34 | 917 | Nerolidol (E-) |
| 18 | 32.19 | 16.98 | 936 | Spathulenol |
| 19 | 33.46 | 1.67 | 780 | Caryophyllene oxide |
| 20 | 33.93 | 7.89 | 932 | Junenol |
| 21 | 35.19 | 3.53 | 901 | Murrolol<epi-alpha-> |
| 22 | 36.6 | 6.69 | 851 | Carotol |
| 23 | 37.62 | 0.35 | 710 | Spathulenol |
| 24 | 38.09 | 0.59 | 840 | Oplopanone |
| 25 | 46.62 | 3.73 | 792 | Androst-2,16-diene |
| 26 | 50.35 | 1.77 | 838 | Phytol |
| 27 | 55.92 | 0.35 | 896 | Tricosane |
| 28 | 56.31 | 2.74 | 927 | Grandiflorenic acid |
| 29 | 58.67 | 0.50 | 893 | Eicosane |

## 7. Characterization of pinostrobin isolated from *Boesenbergia rotunda*

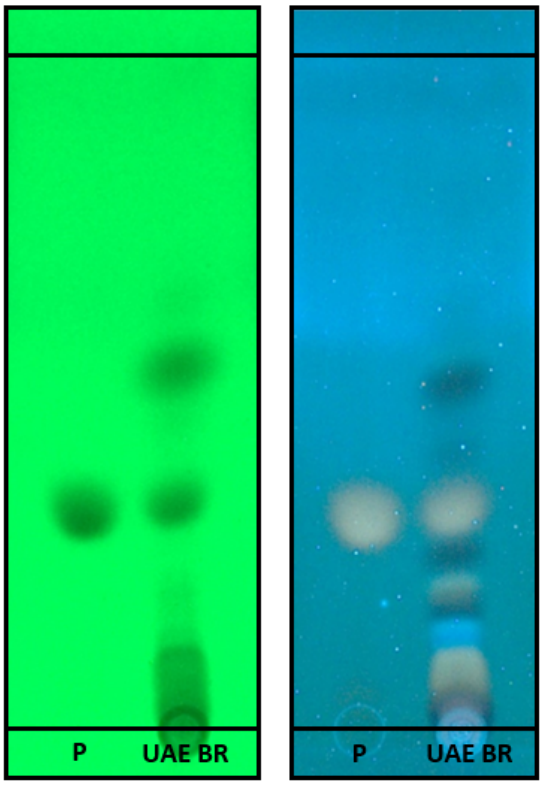



**Figure S4.** Thin layer chromatography of isolated pinostrobin (P) and hexane extract derived from *B. rotunda* (UAE BR) developed in a mobile phase system containing a co-solvent mixture of *n*-hexane:ethyl acetate (9.5:0.5 v/v) and visualization under ultraviolet lights (254 nm; green and 366 nm; blue).

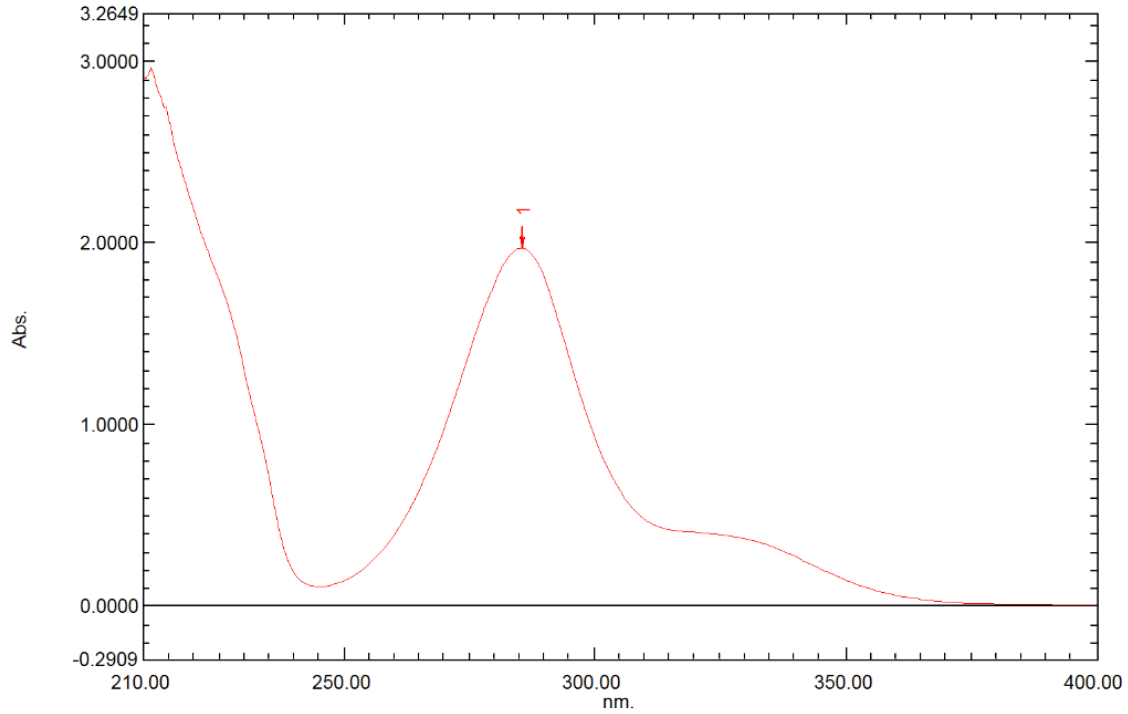



**Figure S5.** UV–Vis spectrum of pinostrobin (in methanol) at the maximum absorbance (285.5 nm).

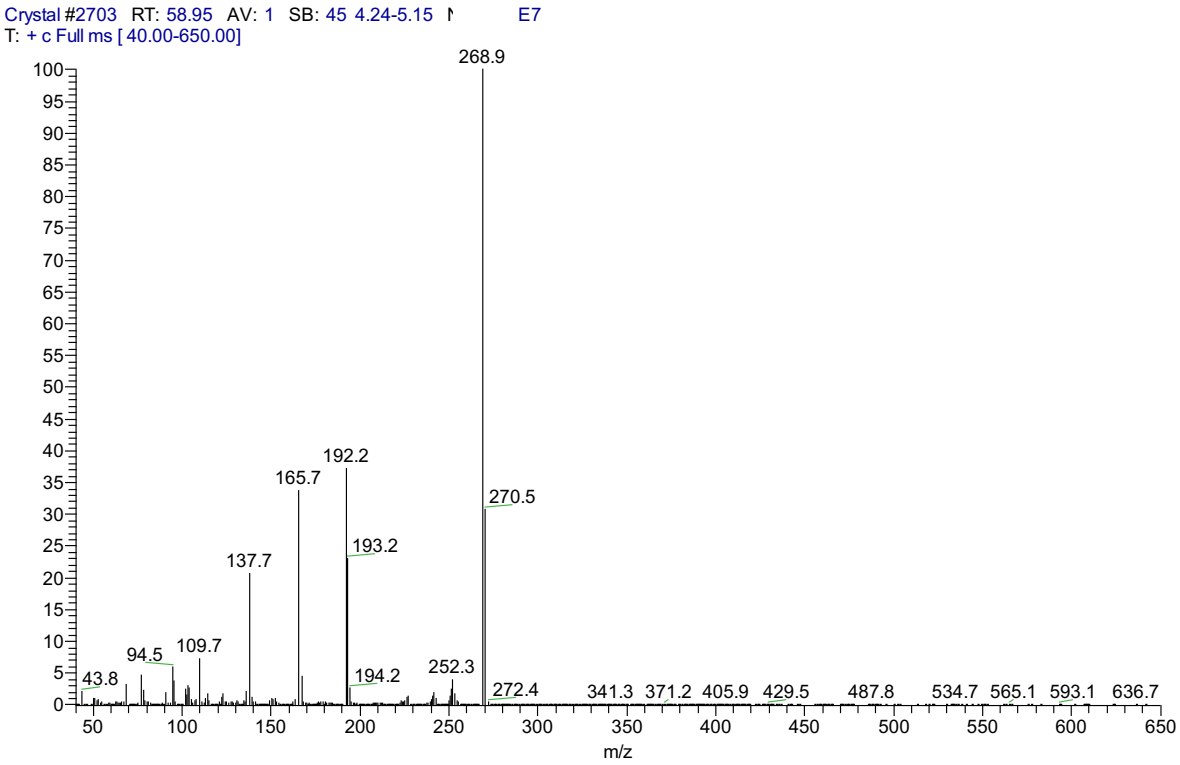
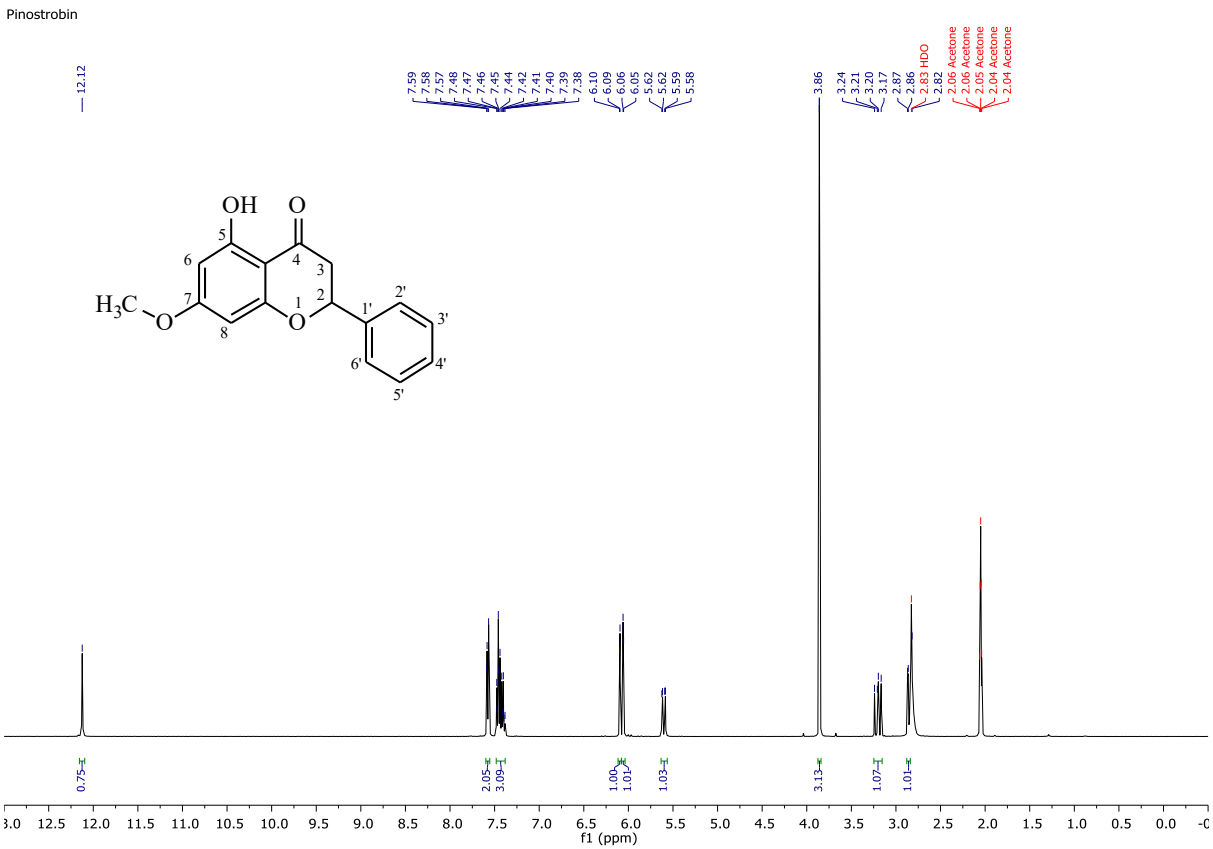



**Figure S6.** Mass spectrometry fragmentation pattern of pinostrobin using gas chromatography–mass spectrometry (GC–MS) with a DSQ quadrupole mass analyzer and an electron impact (EI) ionization positive mode at 70 eV.




$^1$H NMR (400 MHz, Acetone-$d_6$): δ 12.12 (1H, s, 5-OH), 7.59 – 7.57 (2H, m, H-2′, H-6′), 7.48 – 7.38 (3H, m, H-3′, H-4′, H-5′), 6.10 (1H, d, $J$ = 2.3 Hz, H-8), 6.06 (1H, d, $J$ = 2.3 Hz, H-6), 5.60 (1H, dd, $J$ = 3.1, 12.8 Hz, H-2), 3.86 (3H, s, 7-$OCH_3$), 3.20 (1H, dd, $J$ = 12.8, 17.1 Hz, H-$3_{ax}$), 2.85 (1H, dd, overlapping, H-$3_{eq}$).

**Figure S7.** $^1$H-NMR spectrum of pinostrobin (in acetone-d6).

**8. Cyclooxygenase-2 inhibitory activity ($IC_{50}$, μM) of essential oils, hexane extracts, and pinostrobin derived from *B. rotunda***

**Table S7.** Determination of $IC_{50}$ values of pinostrobin derived from *B. rotunda* against human cyclooxygenase-2.

| No | Samples | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | Mean ± SD | Duncan |
| 1 | Pinostrobin | 255.25 | 316.12 | 285.67 ± 43.03 | a |
| 4 | Diclofenac sodium* | 318.74 | 261.96 | 290.35 ± 40.14 | a |
| 5 | BD** | 248.31 | 231.86 | 240.09 ± 11.63 | a |

*Diclofenac sodium is a positive control.
**BD: betamethasone dipropionate is an atopic dermatitis standard drug.

**9. 5-Lipoxygenase inhibitory activity ($IC_{50}$, μg/mL) of essential oils, hexane extracts, and pinostrobin derived from *B. rotunda***

**Table S8.** Determination of $IC_{50}$ vaules of EOs, hexane extracts, and pinostrobin derived from *B. rotunda* against human 5-lipoxygenase.

| No | Samples | $IC_{50}$, μg/mL | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | Mean ± SD | Duncan |
| 1 | Pinostrobin | 0.147 | 0.101 | 0.098 | 0.179 | 0.149 | 0.135 ± 0.035 | a |
| 2 | UAE BR | 11.390 | 12.020 | 11.600 | 12.910 | 9.078 | 11.399 ± 1.423 | c |
| 3 | EO BR | 1.763 | 1.449 | 1.432 | 1.732 | 1.682 | 1.612 ± 0.159 | b |
| 4 | NDGA** | 1.256 | 1.458 | 1.210 | 1.730 | 1.936 | 1.518 ± 0.311 | b |
| 5 | BD*** | 0.847 | 0.936 | 0.895 | 1.315 | 1.246 | 1.048 ± 0.216 | b |

*Note: UAE: hexane extract; EO: essentail oils; and BR: *B. rotunda*; five replicate wells were generated from two rounds of testing. One-way ANOVA was performed to compare the mean of each sample, and different letters indicate the significant difference of the mean.
**NDGA: nordihydroguaiaretic acid is a positive control.
***BD: betamethasone dipropionate is an atopic dermatitis standard drug.

## 10. Steady-state kinetics of 5-lipoxygenase in the presence of inhibitor NDGA and pinostrobin

**Table S9.** Calculated kinetic parameter values of pinostrobin compared to NDGA by the mixed-type inhibition.

| Mixed-type Inhibition | NDGA | Pinostrobin |
|---|---|---|
| $V_{max}$ (M/s) | $7.888 \times 10^{-6}$ | $8.064 \times 10^{-6}$ |
| $K_m$ (μM) | 3.919 | 3.249 |
| $K_i$ (μM) | 3.376 | 2.284 |

*Note: NDGA = nordihydroguaiaretic acid; $V_{max}$ = maximum enzyme velocity; $K_m$ = Michaelis-Menten constant; $K_i$ = inhibition constant.

**Table S10.** Human recombinant 5-lipoxygenase velocity and Michaelis-Menten constant in the presence of NDGA and pinostrobin.

| Substrate concentration (μM) | Control (no inhibitor) | NDGA (μg/mL) 1.56 | NDGA (μg/mL) 3.125 | NDGA (μg/mL) 6.25 | Pinostrobin (μg/mL) 1.56 | Pinostrobin (μg/mL) 3.125 | Pinostrobin (μg/mL) 6.25 |
|---|---|---|---|---|---|---|---|
| 66.67 | 0.0000090934 | 0.00000581159 | 0.0000060934 | 0.00000637037 | 0.00000548 | 0.00000548 | 0.00000559 |
| 33.335 | 0.0000077053 | 0.00000374396 | 0.0000044203 | 0.00000508776 | 0.00000375 | 0.00000417 | 0.00000423 |
| 16.6675 | 0.0000064549 | 0.00000277295 | 0.0000035853 | 0.00000395089 | 0.00000293 | 0.00000325 | 0.00000333 |
| 8.33375 | 0.0000065628 | 0.00000195652 | 0.0000035878 | 0.00000397907 | 0.00000262 | 0.00000297 | 0.00000311 |
| **$V_{max}$ (M/s)** | $9.068 \times 10^{-6}$ | $8.695 \times 10^{-6}$ | $6.364 \times 10^{-6}$ | $6.662 \times 10^{-6}$ | $6.477 \times 10^{-6}$ | $6.073 \times 10^{-6}$ | $6.097 \times 10^{-6}$ |
| **$K_m$ (μM)** | 4.259 | 36.40 | 9.402 | 7.823 | 17.44 | 11.60 | 10.85 |

*Note: NDGA = nordihydroguaiaretic acid; $V_{max}$ and $K_m$ were calculated using the Michaelis-Menten formula in GraphPad Prism v.9.

**11. Molecular modeling of NDGA to human 5-lipoxygenase (PDB ID: 6N2W)**

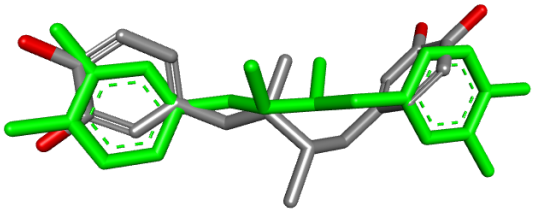


**Figure S8.** Superimposition of the lowest energy conformer of docked nordihydroguaiaretic acid (NGDA) and the co-crystallized NGDA in the 5-lipoxygenase binding site.