The contents determination of rosmanol and carnosol in *Callicarpa longissima*

Chromatographic conditions: The standard reference materials and methanol extract of *Callicarpa longissima* were analyzed by LC-2030C HPLC (SHIMADZU, Japan) with C18 column (250 × 4.6 mm, 5μm) at 30℃. The mobile phase, composed of acetonitrile (A) and 0.1% phosphoric acid (B), was applied in gradient elution model as follows: 0-35 min, 35%A-62%A. The flow rate was kept at 0.8 ml/min and detection wavelength was set at 210 nm.

Preparation of standard reference solution: Rosmanol (4.69 mg) and carnosol (4.04 mg) were dissolved and quantified in 50 ml volumetric flask with methanol.

Preparation of sample solution: 2g powder of branches and leaves of *Callicarpa longissima* was extract with methanol by ultrasonic extractor for 1 h, and the filtered extract solution was quantified in 50 ml volumetric flash with methanol.

Contents determination: The stand curves of rosmanol and carnosol were established and used to calculate for contents based on their peak areas in chromatogram of methanol extract of *Callicarpa longissima*. The results showed that contents of rosmanol and carnosol were 0.46 and 2.37 mg/g, respectively.

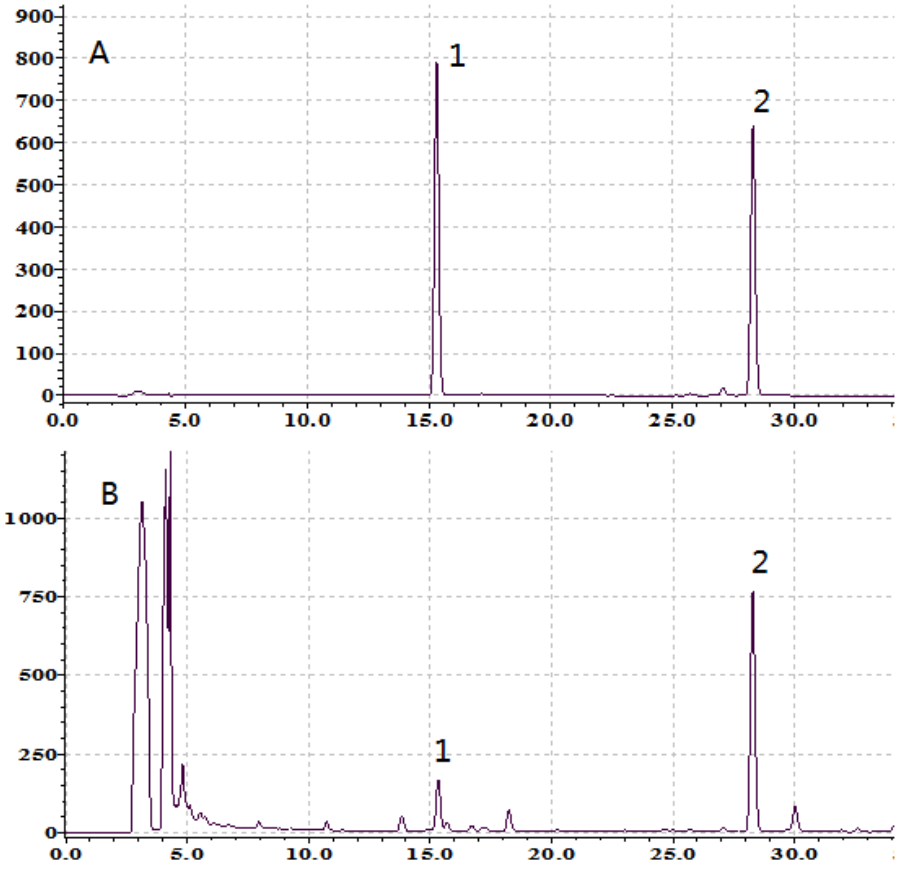

Figure S1. (A) Chromatogram of standard reference materials, in which peak 1, 2 were rosmanol and carnosol, respectively. (B) Chromatogram of methanol extract of *Callicarpa longissima*.

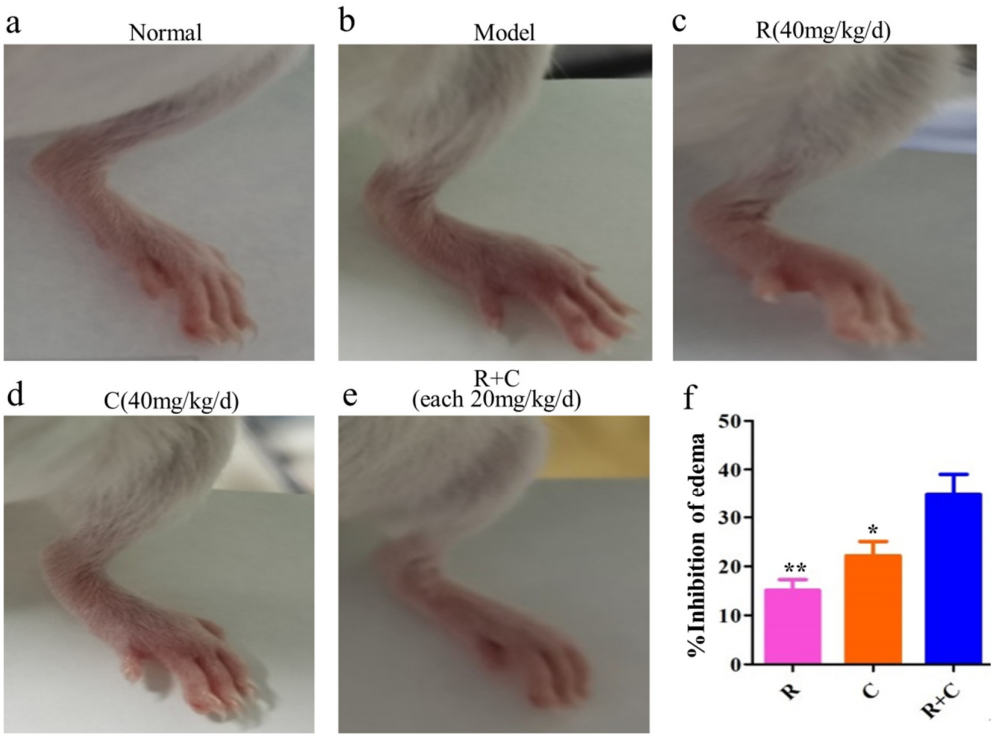

Figure S2. Rosmanol and carnosol synergistically inhibited paw edema in carrageenan-induced Kunming mice model. Paw edema was induced with 1% (w/v) carrageenan suspension by subcutaneous injection. Rosmanol (40 mg/kg), carnosol (40 mg/kg) and their combination (20 mg/kg rosmanol and 20 mg/kg carnosol) were administered by intragastric gavage 1 h before subcutaneous injection. Representative pictures from the left hind limb of the normal group (a), model group (b), rosmanol-treated group (c), carnosol-treated group (d) and combination-treated group (f). Inhibitory rate of rosmanol (R), carnosol (C) and their combination (R+C) on paw edema (f). Data were presented as the mean ± sd (n = 6). The significance of differences was analyzed by unpaired t-test. *, $p<0.05$ *vs* the combination-treated group; **, $p<0.01$ *vs* the combination-treated group.

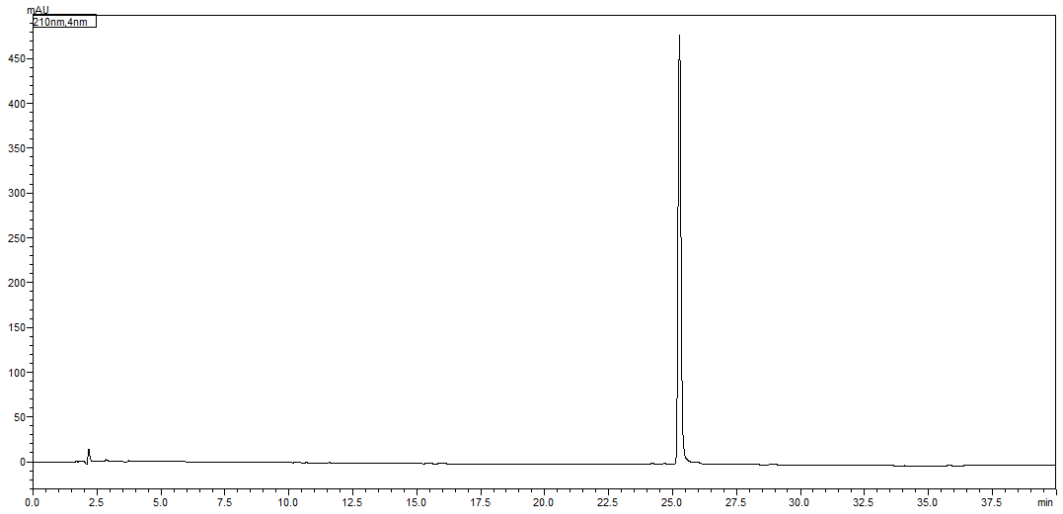

Figure S3. HPLC spectrum of rosmanol (**1**).

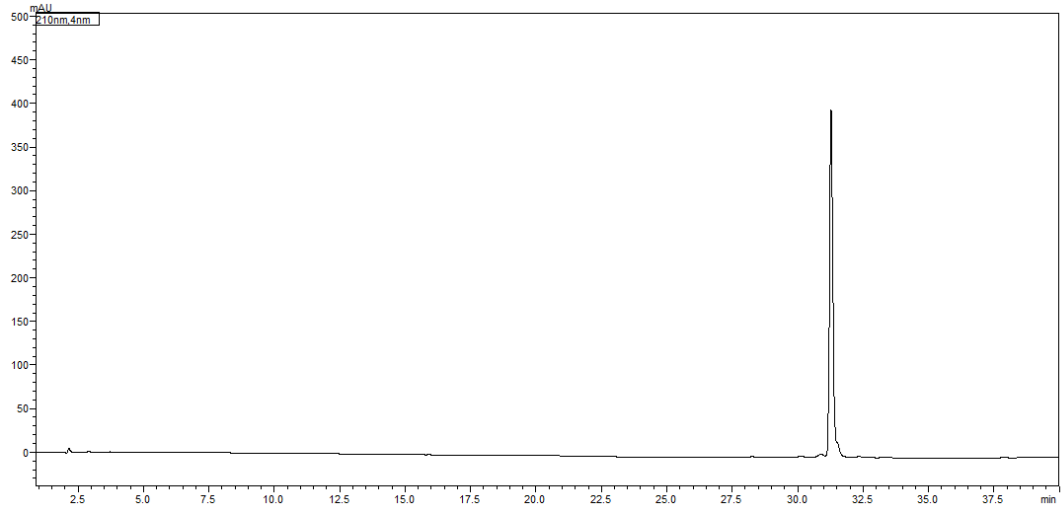

Figure S4. HPLC spectrum of carnosol (**2**).

Structure elucidation

Compound **1,** obtained as white powder, had the molecular formula $C_{20}H_{26}O_5$ on basis of HR-ESI-MS at m/z 345.1688 [M-H]$^-$. ^{1}H-NMR (500 MHz, CD_3OD), δ_H: 6.84 (1H, s, H-14)，4.59 (1H, d, J = 3.5 Hz, H-6α), 4.52 (1H, d, J = 3.5 Hz, H-7β), 3.29 (1H, m, H-1β), 3.19 (1H, m, H-15), 2.26 (1H, s, H-5), 1.94 (1H, td, J = 14.0, 5.5 Hz, H-1α), 1.60 (1H, m, H-2β), 1.44 (2H, m, H-2α, 3β), 1.23 (1H, td, 13.5, 3.0 Hz, H-3α), 1.21 (3H, d, J = 7.0 Hz, H-16), 1.18 (3H, d, J = 7.0 Hz, H-17), 1.03 (3H, s, H-18), 0.91 (3H, s, H-19); ^{13}C-NMR (125 MHz, CD_3OD), δ_C: 28.7 (C-1), 20.2 (C-2), 39.5 (C-3), 32.4 (C-4), 51.7 (C-5), 80.0 (C-6), 69.3 (C-7), 129.5 (C-8), 125.1 (C-9), 48.4 (C-10), 145.4 (C-11), 143.5 (C-12), 137.5 (C-13), 120.5 (C-14), 28.0 (C-15), 23.0 (C-16), 23.2 (C-17), 31.9 (C-18), 22.5 (C-19), 181.0 (C-20). The NMR spectral data of **1** were in good agreement with those of rosmanol reported in the reference, so was identified as rosmanol.

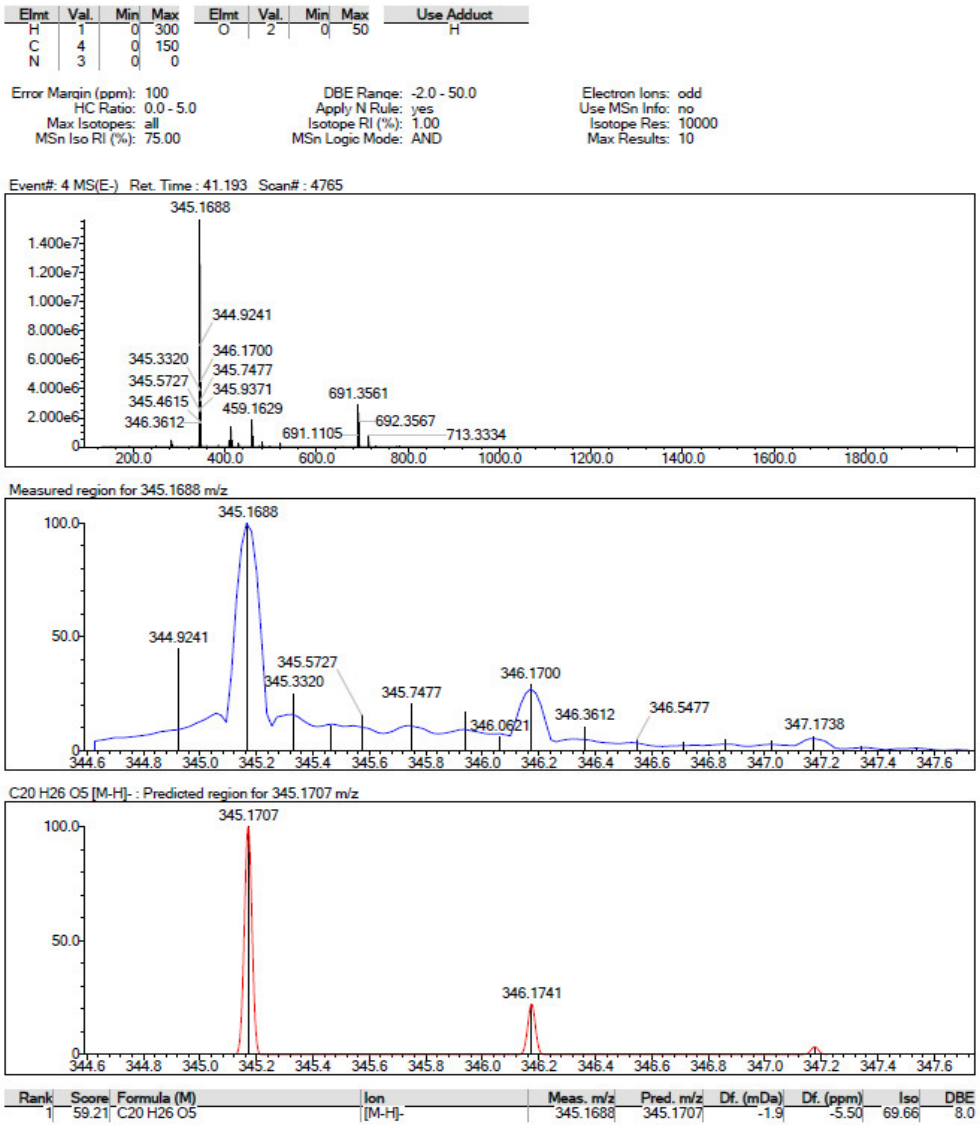

Figure S5. HR-ESI-MS spectrum of rosmanol (**1**).

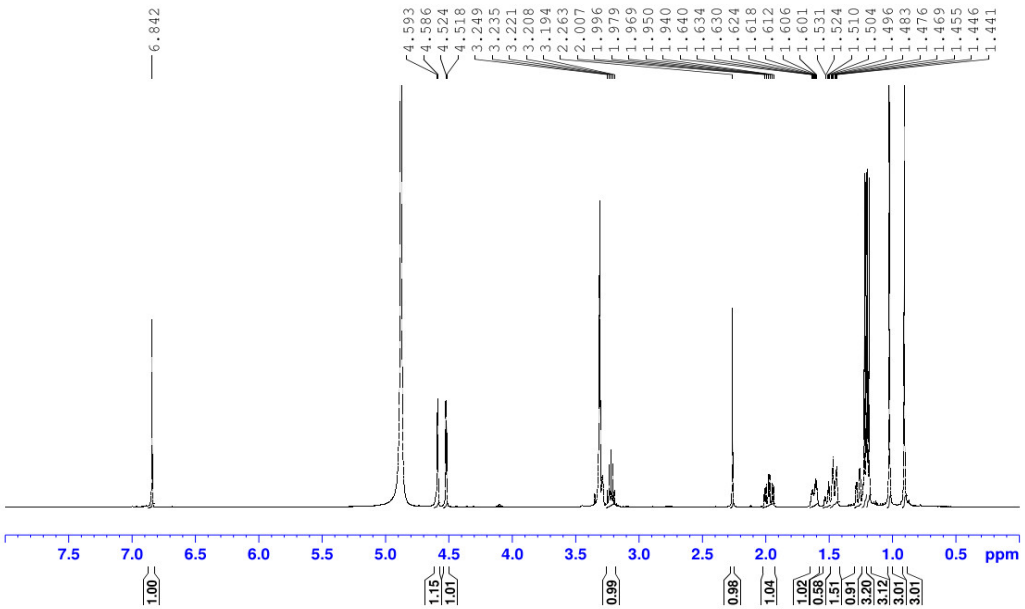

Figure S6. ^{1}H-NMR spectrum of rosmanol (**1**) (500 MHz in CD_3OD).

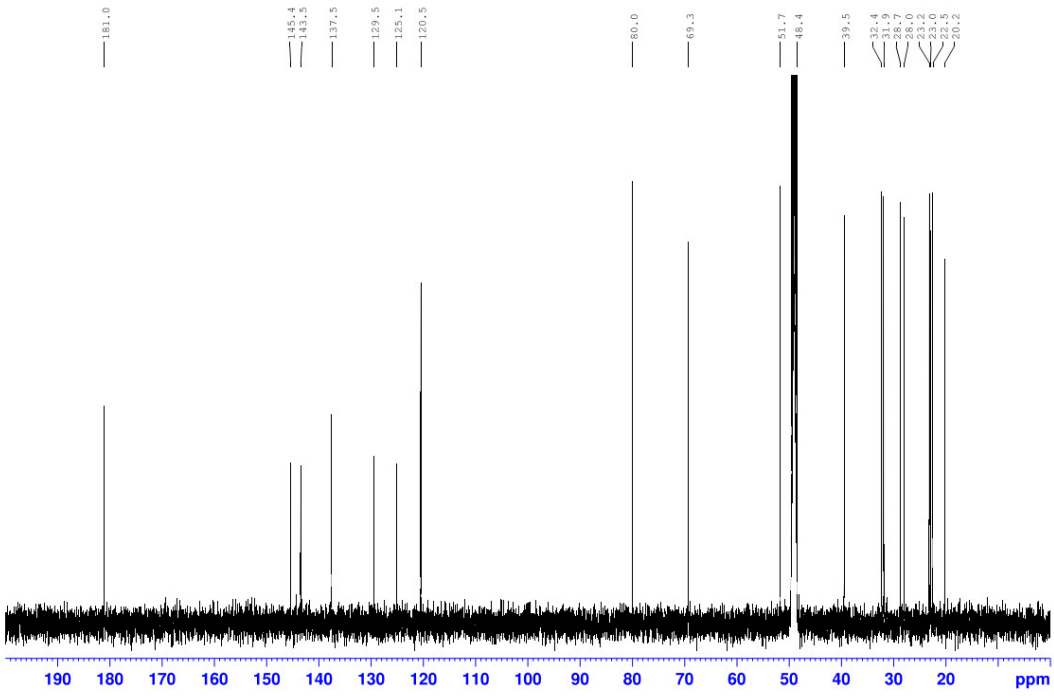

Figure S7. ^{13}C-NMR spectrum of rosmanol (125 MHz in CD_3OD).

Compound **2**, obtained as white powder, had the molecular formula $C_{20}H_{26}O_4$ on basis of HR-ESI-MS *m/z* 329.1745 $[M-H]^-$. ^{1}H-NMR (500 MHz, CD_3OD), δ_H: 6.69 (1H, s, H-14), 5.42 (1H,

d, J = 2.0 Hz, H-7), 3.22 (1H, m, H-15), 2.79 (1H, br d, J = 14.5 Hz, H-1β), 2.54 (1H, td, J = 14.0, 4.5 Hz, H-1α), 2.18 (1H, m, H-6α), 1.83 (2H, m, H-2β, 6β), 1.68 (1H, dd, J = 11.0, 6.0 Hz, H-5), 1.61 (1H, m, H-3β), 1.51 (1H, m, H-2α), 1.31 (1H, td, J = 13.5, 3.5 Hz, H-3α), 1.19 (3H, d, J = 7.0 Hz, H-16), 1.18 (3H, d, J = 7.0 Hz, H-17), 0.88 (3H, s, H-18), 0.87 (3H, s, H-19). ^{13}C-NMR (125 MHz, CD_3OD), δ_C: 30.1 (C-1), 20.0 (C-2), 42.2 (C-3), 35.5 (C-4), 47.0 (C-5), 30.9 (C-6), 79.7 (C-7), 133.3 (C-8), 123.0 (C-9), 49.8 (C-10), 144.7 (C-11), 144.2 (C-12), 136.1 (C-13), 112.5 (C-14), 28.0 (C-15), 23.2 (C-16), 20.1 (C-17), 32.2 (C-18), 23.2 (C-19), 179.3 (C-20). The NMR spectral data of **2** were in good agreement with those of carnosol reported in the reference, so was identified as carnosol.

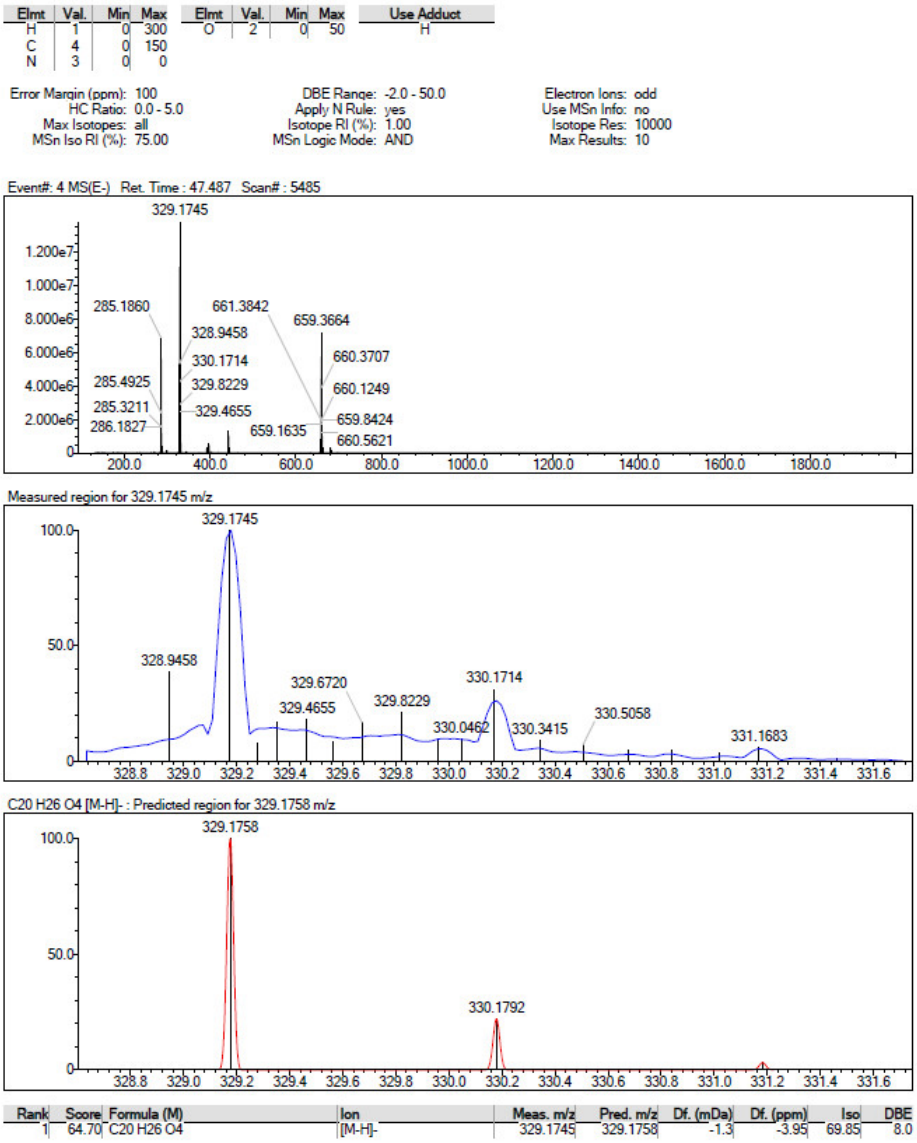

Figure S8. HR-ESI-MS spectrum of carnosol (**2**).

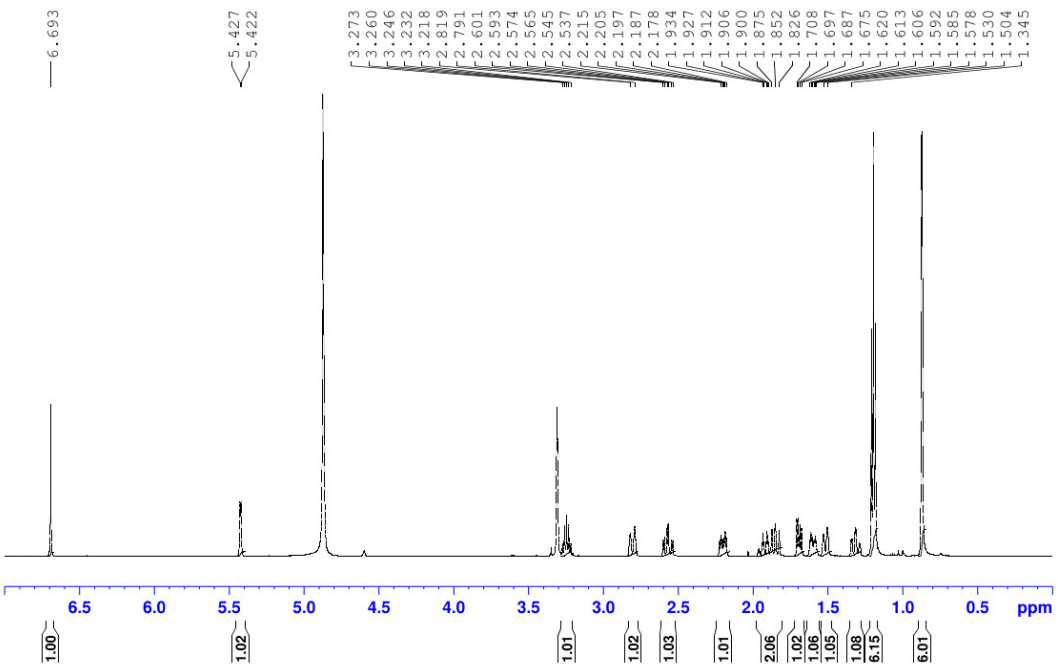

Figure S9. ^{1}H-NMR spectrum of carnosol (**2**) (500 MHz in CD_3OD).

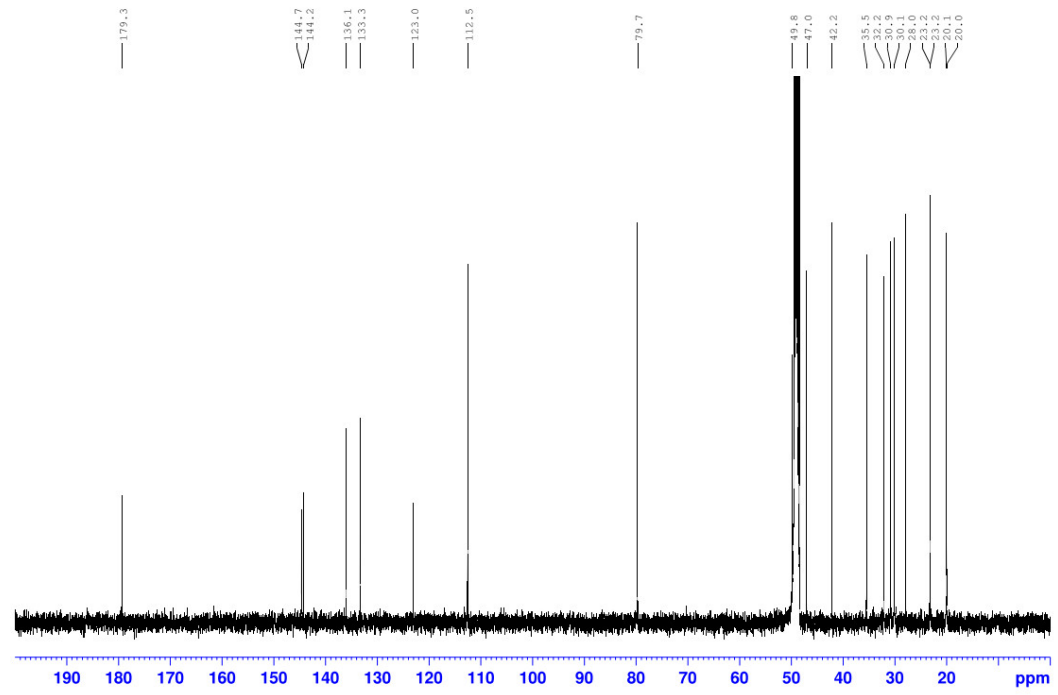

Figure S10. ^{13}C-NMR spectrum of carnosol (**2**) (125 MHz in CD_3OD).

Table S1

Effects of rosmanol, carnosol and their combination on body weight in CIA DBA/1 mice

Groups	Body weight (g, n=6)				
	d0	d21	d28	d35	d42
Normal	18.9±0.7	21.7±0.8	22.5±1.0	23.4±1.2**	24.1±1.3**
CIA model	19.0±0.6	21.5±0.9	21.3±0.9	21.0±1.0	20.8±1.2
R	18.8±0.7	21.4±0.8	21.5±0.9	21.5±0.9	21.7±1.0
C	18.7±0.7	21.4±0.8	21.6±1.0	21.9±1.1	22.1±1.2
R + C	19.1±0.6	21.5±0.9	21.8±1.1	22.3±1.0*	22.7±1.3*

Compare to CIA model group,* $p<0.05$, **$p<0.01$.