Supporting information

Acetyl shikonin induces IL-12, nitric oxide and ROS to kill intracellular parasite *Leishmania donovani* in infected hosts.

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S1. Isolation of Acetyl shikonin: Adsorption based elution

Solvent extract was prepared from authenticated plant material (root bark) of *A. nobilis*. The best adsorbent was selected based on a preliminary adsorption experiment with solvent extract. The adsorption isotherm models with single solute (AS) and multi-solute (HE) were compared and differences in adsorption capacities were observed. Activated carbon (10 g/l) (particle size: 200-325 mesh size; surface area: 750 m^2/g) was added to solution containing 500 mg of solvent extract solubilised in 100 ml of 30% isopropanol in water (v/v). The experiment was maintained at room temperature with agitation of 100 rpm for 90 min. After equilibration, excess solution was aspirated out and pure water was added. Separation of pigment molecules was done by addition of increasing concentrations of IPA in water (10-100% [v/v]). The contact time for desorption was maintained as 30 min. The pigment separated in different IPA/Water ratio was analyzed through High Performance Thin Layer Chromatography (HPTLC).

Based on preliminary observations, activated charcoal (Particle size: 200-325, surface area: 750 m^2/g) was selected to adsorb the pigments and then selectively desorb with 70% IPA/water (v/v). The adsorption mechanism was found to follow Langmuir model as analyzed by isotherm models. Optimization by response surface modelling by Box-Behnken design depicted that the amount of adsorbent (8.93 g/l), desorption time (31 min), IPA (72 % v/v in water) at agitation of 150 rpm as optimized values yielded 5.25 mg/g of plant dry material. The pure acetyl shikonin obtained was characterized using spectral techniques and used directly for further studies.

Acetyl Shikonin : $C_{18}H_{18}O_6$; red powder; $[\propto]_D^{20}$ = +456 (c 0.1, CHCl3); mp: 104-106 °C; ESI-MS m/z = 329[M-1] FTIR (ν_{max}^{KBr}) 3401, 2364, 1742, 1613, 1231, 1050 cm^{-1}.

^{1}H-NMR (400MHz, CDCl3): δ 12.56 (s, 1H, –OH,**H-18**), 12.40 (s, 1H, –OH, **H-17**), 7.19(s, 2H, **H-6,7**), 6.98 (d, J = 1.2 Hz, 1H, **H-2**), 6.03 (ddd, J = 1.2, 4.6, 7.2 Hz, 1H, **H-11**), 5.13 (t,

J = 7.26 Hz, 1H,**H-13**), 2.59 (m, 1H, **H-12a**), 2.46(m, 1H,**H-12b**), 2.13 (s, 3H, CO$\underline{CH_3}$-**H-19**), 1.69 (s, 3H, $\underline{CH_3}$-**H-15**), 1.57 (s, 3H, $\underline{CH_3}$-**H-16**).

^{13}C-NMR (100MHz, CDCl3): δ 178.32(**C-1**), 176.82(**C-4**), 169.87(**C-20**), 167.61(**C-8**), 167.08(**C-5**), 148.35(**C-3**), 136.23(**C-14**), 132.84(**C-6**), 133.00(**C-7**), 131.84(**C-2**), 117.82(**C-15**), 111.70(**C-9**), 111.96(**C-10**), 69.65(**C-11**), 32.97(**C-12**), 25.88(**C-15**), 21.06(**C-19)**, 18.06(**C-16**).

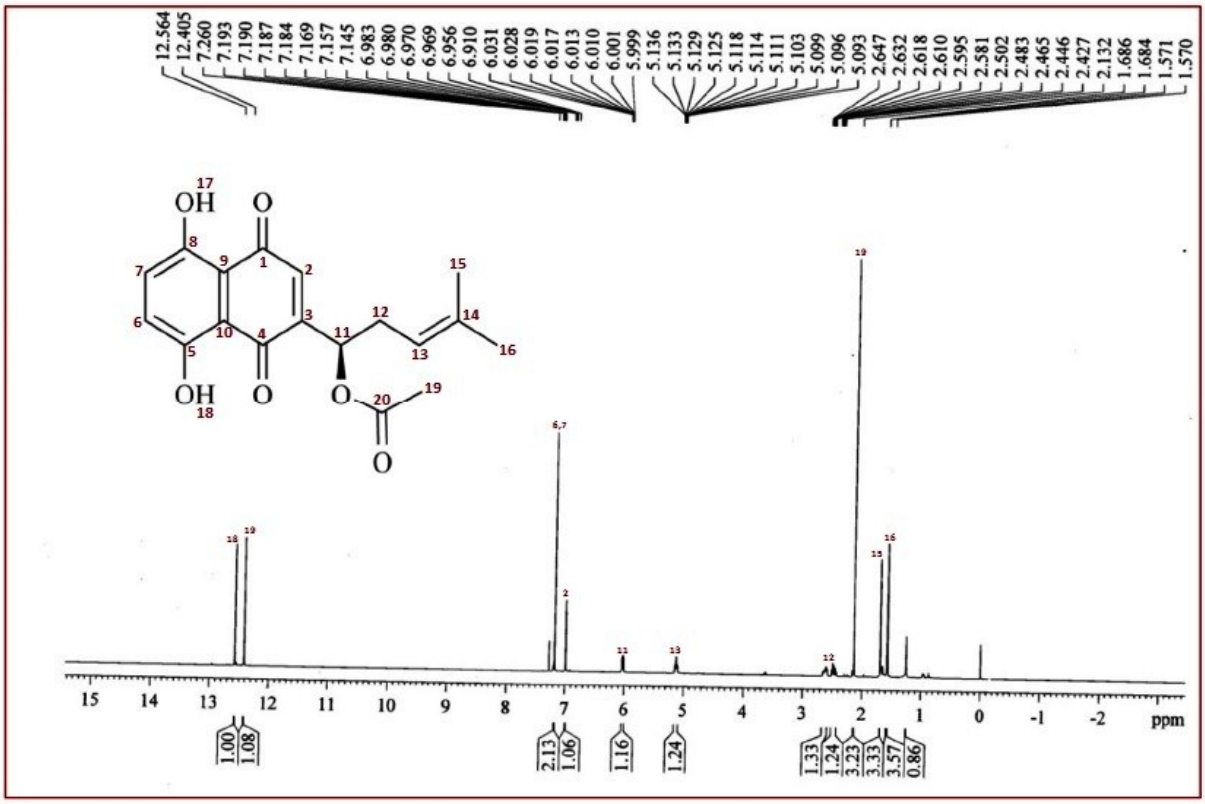

Figure S1: Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra of Acetyl Shikonin.

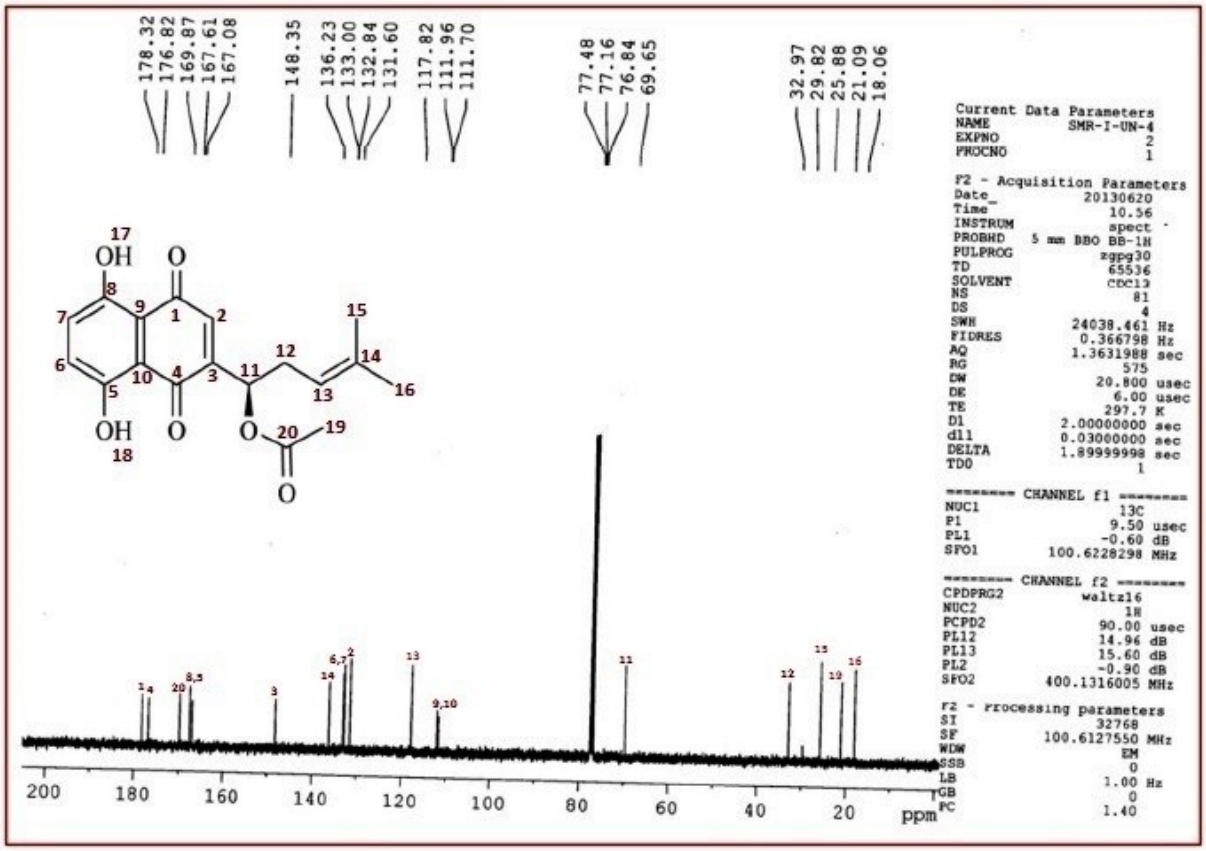

Figure S2: Carbon Nuclear Magnetic Resonance (^{13}C-NMR) spectra of Acetyl shikonin.

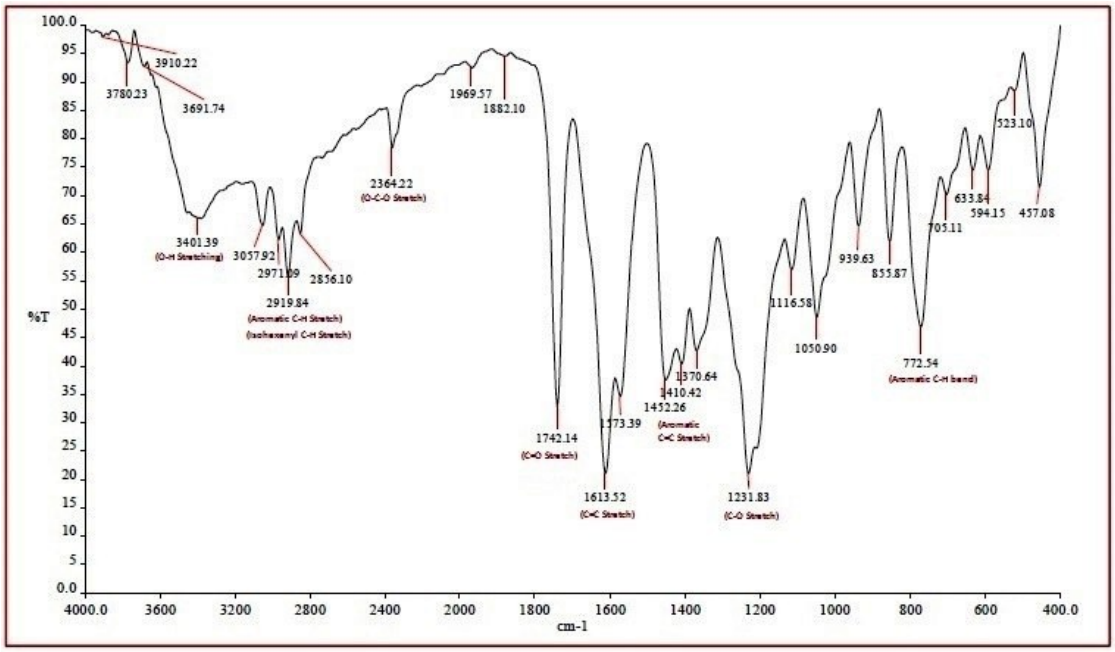

Figure S3: Fourier Transformed Infrared Spectra (FT-IR) of Acetyl Shikonin.

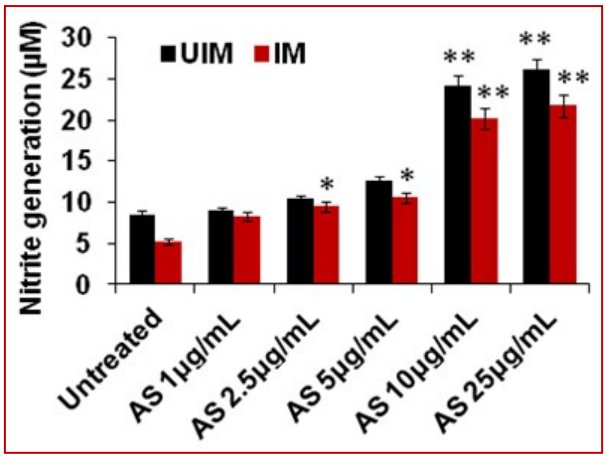

Figure S4: Concentration dependent acetyl shikonin (AS) induced nitrite generation in uninfected (UIM) and *L. donovani*-infected macrophages (IM).

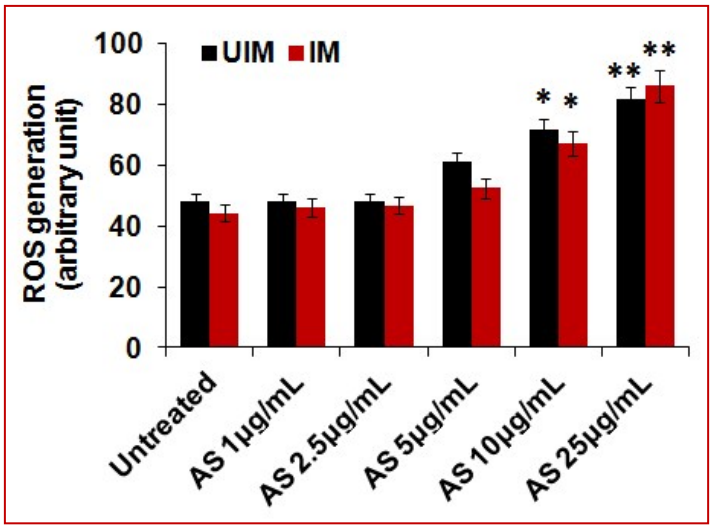

Figure S5: Concentration dependent acetyl shikonin (AS) induced ROS generation in uninfected (UIM) and *L. donovani*-infected macrophages (IM).