Figure 3: TNF-α release from non-activated N9 microglial cells following a 24 h incubation with the bacopa extracts. Neither the infusion (A), or alkaloid (C) fractions or the Bacoside A (D) induces an increase in the release of TNF-α from the microglial cells compared to media controls. The highest concentration of the tea extract (B) results in a small but significant release of TNF-α compared to the media control. Graphs represent mean ± SEM. *$p<0.05$; ANOVA with Dunnett's post hoc test compared to media control.

Figure 4: IL-6 release from LPS-activated N9 microglial following a 24 h incubation with LPS plus the bacopa extracts. The infusion extract significantly inhibited the release of IL-6 from LPS activated microglial at 25 and 50 μg compared to LPS controls (A). The highest concentration of the alkaloid fraction (1 μg) also inhibited the release of IL-6 from activated microglia (C). Neither the tea nor the Bacoside A significantly inhibited IL-6 release compared to LPS controls (B and D). Data is normalized to IL-6 release from LPS activation only. Graphs represent mean ± SEM. *$p<0.05$; ANOVA with Dunnett's post hoc test compared to LPS control.

Figure 5: TNF-α release from LPS activated N9 microglial cells following a 24 h incubation with bacopa plus the extracts. Both the infusion (A) and the alkaloid (C) fraction showed significant inhibition of the release of TNF-α from LPS activated microglia of approximately 20%-27% compared to LPS alone. The tea extract (B) did not inhibit the release of TNF-α. The Bacoside A (D) showed a small but significant increase in TNF-α release at the highest concentration. Data is normalized to TNF-α release from LPS activation only. Graphs represent mean ± SEM. *$p<0.05$; ANOVA with Dunnett's post hoc test compared to LPS control.

Figure 6: Each bacopa extract was evaluated for its ability to inhibit caspase-1, caspase-3, and MMP-3 in 96 well assays. These data are expressed in terms of % inhibition of enzyme activity. Control 1 included the substrate and enzyme (no inhibitor) and Control 2 included the substrate, enzyme and a specific inhibitor (as described).

Supplemental Figure 1: High performance thin layer chromatography (HPTLC) identification of the *Bacopa monnieri* that was obtained from Banyan Botanicals. Covance performed the analysis and provided a Certificate of Analysis for the Banyan material. A) Visible light HPTLC. Note the test and reference materials show numerous brown zones between Rfs 0.24 and 0.85. B) UV Light 365 nm HPTLC. The test and reference materials show numerous brown zones between Rfs 0.23 and 0.72. They also show bright blue/red zones around Rf 0.84. Lane 1 is a solvent blank. Lane 2 is *Bacopa monnieri* (ChromaDex standard, lot 30842-197 exp April 2012). Lanes 3 and 4 are *Bacopa monnieri* from Banyan Botanicals that was used in the current experiments.

References

Aguiar, S., Borowski, T., 2013. Neuropharmacological review of the nootropic herb Bacopa monnieri. Rejuvenation research 16, 313-326.