# Methyl Syringate as a Potential Biomaterial Additive: A Honey-Derived Compound with Strong Antioxidant and Anti-Inflammatory Capabilities

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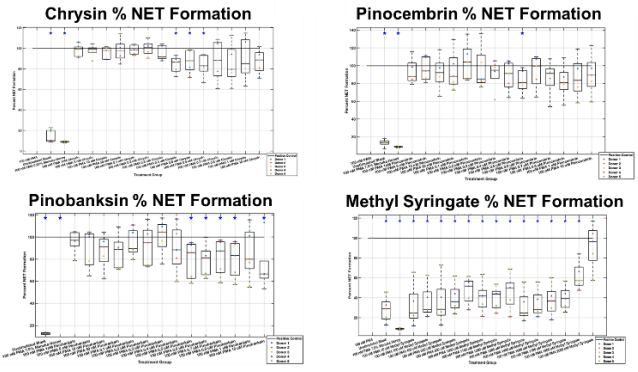
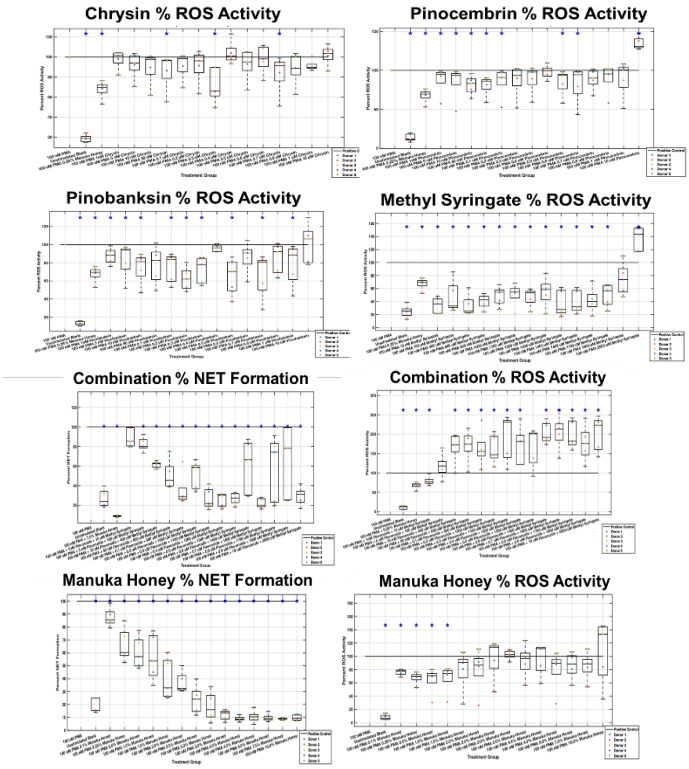
**Statement of Purpose:** Neutrophils use reactive oxygen species (ROS) activity and a specialized form of cell death named NETosis to kill and ensnare invading pathogens. While these neutrophil behaviors are critical in preventing infection, a dysregulated response can be deleterious and lead to tissue damage and fibrosis at host-biomaterial interfaces. Manuka honey has demonstrated potent antibacterial properties and recently, anti-inflammatory potential. It was hypothesized that applying the flavonoids found in Manuka honey: chrysin, pinocembrin, pinobanksin, and the phenolic compound methyl syringate to neutrophils exhibiting pro-inflammatory behavior will reduce ROS activity and prevent NETosis in primary human neutrophils. Additionally, it was investigated whether these compounds act more effectively as isolates or synergistically within whole Manuka honey. Thus, concentrations of whole Manuka honey were also assayed as well as all four individual compounds in combination

**Methods:** Neutrophils were isolated from whole human blood using a lab-validated procedure involving density sedimentation. Isolated neutrophils were seeded at 100,000 cells/well in 150 µL HBSS (without calcium and magnesium), 0.2% autologous serum, and 10mM HEPES. Manuka honey component or whole Manuka honey concentrations were added, and intracellular ROS and NETosis were stimulated with 100 nM PMA. For NETosis, neutrophils were stained with 0.25 µM Sytox orange (Figure 3b-e) at terminal timepoints to stain extracellular NET DNA measured at 3 and 6 hours via spectrophotometer (Excitation/Emission 540/580). DCFH-dyed primary Intracellular ROS activity was determined via spectrophotometer at an Excitation/Emission of 485/535 at 3 and 6 hours. Results were expressed as relative ROS activity or NET formation (percentage) compared to positive control (stimulated cells) with standard deviation (n=5). Data were pooled, tested for normality, and statistically compared to positive control using Wilcoxon rank sum tests.

**Results:**




**Conclusions:** When compared to positive control levels, individual flavonoids showed small effect sizes, and surprisingly, higher concentrations of flavonoids stimulated ROS. Whole Manuka honey reduced NET levels by up to 91% in step-wise increasing pattern across all concentrations, but only reduced ROS activity by 36% in a narrow concentration range. Methyl syringate, however, reduced NET levels by up to 68% and ROS activity by 66% within a broad therapeutic window of 10-1700 µM. The combination of all flavonoids and methyl syringate reduced NET levels in a similar trend to methyl syringate alone, but sharply increased ROS activity. Manuka honey was most effective in reducing NET formation, bringing NET levels to near baseline unstimulated values, yet fell short of methyl syringate in terms of ROS activity reduction.

**References:**

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