K50 A Forensic Characterization of Bacterial and Fungal Organisms in Traditional Chinese Herbs

Julia Grzymkowski, 1200 W Marshall Street, 242A, Richmond, VA 23220; Justin L. Poklis, BS, Virginia Commonwealth University, Dept of Pharmacology & Toxicology, 410 N 12th Street, Rm 746, PO Box 980613, Richmond, VA 23219-0613; Christopher J. Ehrhardt, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284; and Michelle R. Peace, PhD, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284*

After attending this presentation, attendees will be aware of the potential presence of toxic microorganisms in Chinese herbal products used for medicinal purposes.

This presentation will impact the forensic science community by potentially being used to identify unknown specimens of herbs, provide provenance of the herbs themselves, and help reconstruct toxicological episodes that result in medical emergencies or death.

Traditional Chinese Medicine (TCM) is one of the oldest healing methods used in Chinese culture, often referred to as "formula." TCM's herbal formulations are prescribed for a variety of illnesses, based on symptoms presented. Herbal remedies are selected based on Chinese ancient literature and individual experiences of patients or doctors. Use of TCM in the United States has increased because herbal remedies are believed to be less expensive and more effective with less adverse effects in comparison to traditional pharmaceutics. Therefore, sales have increased, despite articles and case studies that have demonstrated the dangers, such as injury and death, related to TCM, stemming from improper labelling, toxic contaminants, and, in some cases, the presence of pathogenic bacteria.

Objective: The purpose of this study was to conduct a molecular and biochemical survey of microorganisms of 11 Chinese herbal products purchased from a traditional medicine shop in Beijing, China. Bulk analysis of microbial/fungal lipids from the herbs was conducted using a rapid method for extraction of cellular fatty acids and derivatization into Fatty Acid Methyl Esters (FAMEs) prior to profiling with Gas Chromatography/Flame Ionization Detector (GC/FID).

Methods: Eleven over-the-counter Chinese herbs were purchased from Tong Ren Tang in Beijing, China. These herbs were chosen based on reported pharmacological activity: sedative and hypnotic (Suan Zao Ren, Fu Ling, Sha Yuan Zi, and Di Long); anticonvulsant (Gou Teng, Tian Ma, and Jiang Can); and analgesic (Yan Hu Suo, Chuan Duan, Wu Yao, and Mo Yao).

Approximately 100mg of the herbal product was placed in 1x Phosphate Buffered Saline (PBS). A 100µL aliquot of the solution was spread onto Tryptic Soy Agar (TSA) with and without blood supplements (50mg/L). Plates were then incubated overnight at 30°C. Colony growths were photographed, harvested, and subjected to FAME profiling.

FAME profiling of the herbal products was performed using GC/FID equipped with a series of analytical standards to detect and quantify fatty acids between 9 and 20 carbons in length.

Products were incubated with methanolic potassium hydroxide (5% KOH, 95% CH_3OH). The methyl esters were then extracted into hexane and analyzed. The individual fatty acids were identified by their retention time through comparison to reference standards.

Results: Strains within the *Bacillus* group were identified in nearly all 11 of the herbal samples. These included *B. subtilis* and *B. cereus*, as well as *B. megaterium*, *B. circulans*, and *B. atrophaeus*. Organisms belonging to the *Bacillus* ACT group (anthracis, cereus, thuringiensis) were identified in 5 out of 1 herb cultures as evidenced by the large ratio of 15:0 iso to 15:0 anteiso fatty acid biomarkers. A gram-positive, aerobic bacteria related to the *Bacillus* group, *Paenibacillus thiaminolyticus*, was also detected. This bacteria differs from *Bacillus* ACT and has been reported to cause bacteremic infections in humans. All of the herbal specimens also exhibited fungal biomarkers such as polyunsaturated 20:4 ω6,9,12,15c, and 18:3 ω6c (6,9,12). The presence of fungal biomarkers would be consistent with the origin of some herbal samples such as Jiang Can, silkworm larvae that are claimed to have been killed with the fungus *Beauveria bassiana*; however, in others, they could represent contamination of fungal spores.

Conclusion: The characterization of microorganisms present in these traditional Chinese herbs was successful through analysis of their FAME profiles and by the presence of particular and unique fatty acids. The bacterial and fungal identification can potentially be used to identify unknown specimens, provide provenance of the herbs themselves, and help to reconstruct toxicological episodes that result in medical emergencies or death.

Chinese Herbs, FAME Analysis, GC/FID

*Presenting Author