



2018

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Grzymkowski, Julia; Ehrhardt, Christopher J.; Poklis, Justin L.; and Peace, Michelle R., "The Forensic Characterization of Bacterial and Fungal Organisms in Traditional Chinese Medicine" (2018). *Undergraduate Research Posters*. Poster 274.
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Forensic Characterization of Bacterial and Fungal Organisms in Traditional Chinese Medicine

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Purpose

The first aim of this study was to perform a survival experiment to demonstrate the importance of proper herbal brewing technique. The second aim was to conduct a molecular and biochemical survey of microorganisms present on eleven Chinese herbal samples through Fatty Acid Methyl Ester (FAME) analysis with identification of FAMES done using Gas-Chromatography coupled to a Flame Ionization Detector (GC-FID).

Introduction

Traditional Chinese Medicine



- Dates back to 2800 BC
- 6,926 plants, 23,033 chemical substances
- Rx based on Ch. literature and experience
- Dietary Supplements
- Estimated 50% of U.S. population consume
- Dietary Supplement Health and Education Act of 1994
- Recent dangers
- Improper labeling
- Toxic contaminants; pathogenic bacteria

Forensic Science International 241 (2014) 138–140
 Contents lists available at ScienceDirect
Forensic Science International
 ELSEVIER
 journal homepage: www.elsevier.com/locate/forensicint

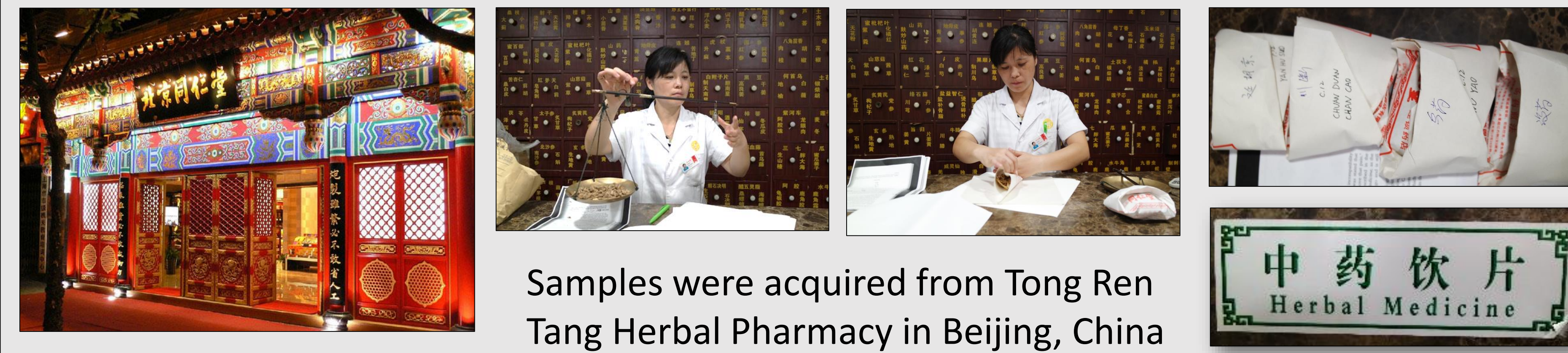
Lethal hepatocellular necrosis associated with herbal polypharmacy in a patient with chronic hepatitis B infection
 John D. Gilbert^a, Ian F. Musgrave^b, Claire Hoban^b, Roger W. Byard^{a,b,*}

- Case History
- 43 year old woman with IBS
- Consulted a traditional Ch. herbal doctor
- Received a variety of herbs
- Flu-like symptoms
- Liver and renal failure
- Multi-organ failure in surgery
- Pure growth of *Klebsiella pneumoniae* from lung tissue
- Herbs prescribed in common with study:
 - *Astragalus sp.*
 - *Poria cocos*
- Conclusions
 - Most dangerous strategy of taking herbal medicines
 - Species is a common contaminant, along with *Bacillus sp.*

Bacterial Genus of Interest: *Bacillus*

- *Bacillus cereus* T-strain
 - Human food-borne pathogen.
 - Produces toxins causing diarrhea, nausea, and vomiting.
 - Forms biofilms
- *Bacillus anthracis*
 - Releases edema toxin which upsets cell water homeostasis, resulting in massive edema.
 - Skin lesion -> papule -> black eschar

Samples



Samples were acquired from Tong Ren Tang Herbal Pharmacy in Beijing, China

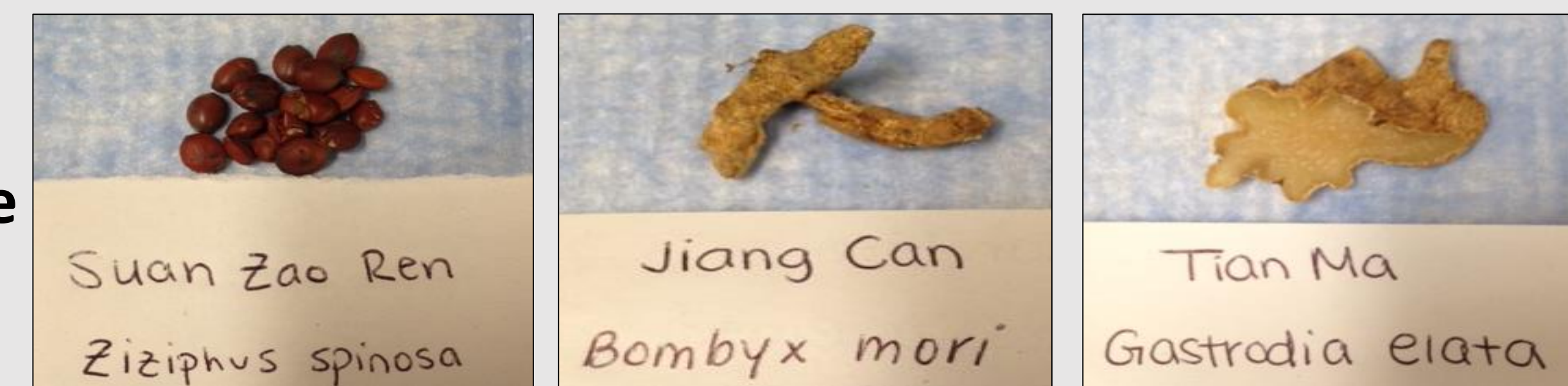
Sedative/Hypnotic



Analgesic



Anticonvulsive



Survival Study

2.80 g of herb mixture + 500 µL liquid *Bacillus cereus* T-strain spores (2.4 mg dried)
 Frozen then lyophilized [n=3]
 After each cooking method, liquid was collected and plated on TSA

Method translated from package

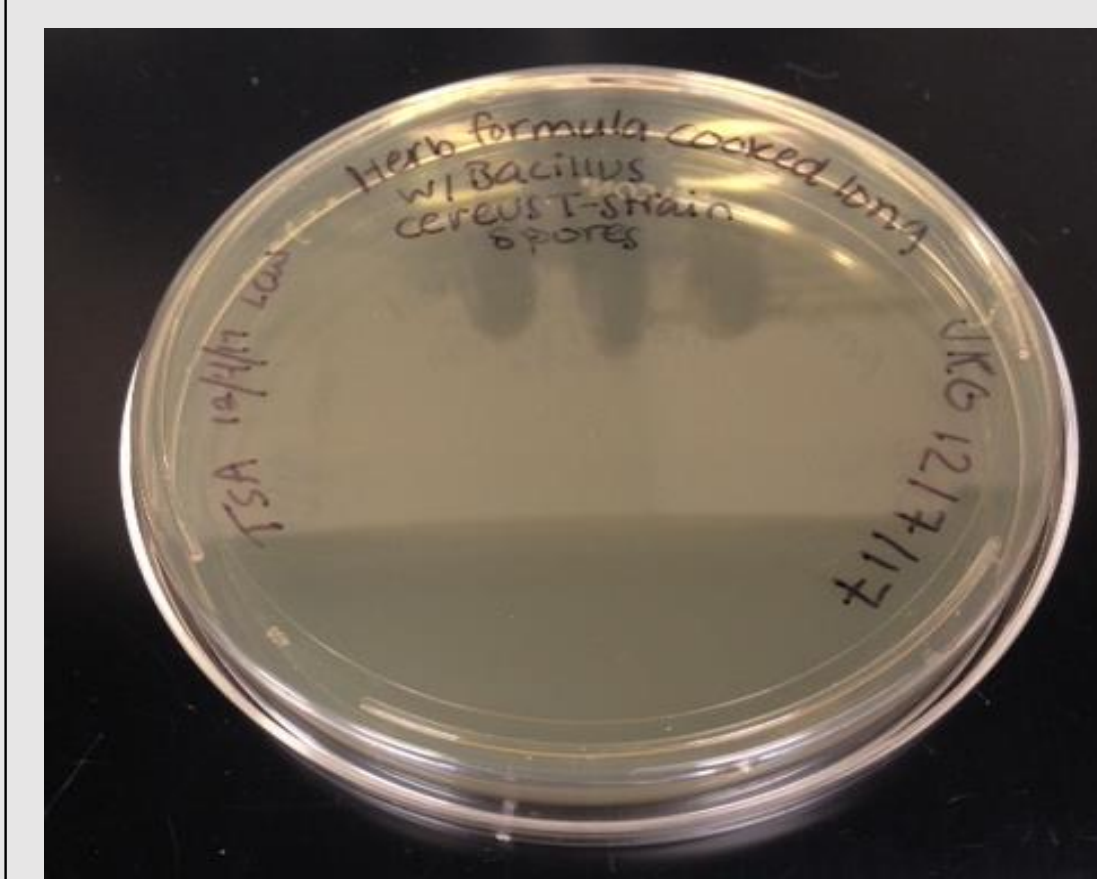
Soaked in cold water (30 mins)
 Cooking:
 Boiling heat (10 mins)
 Low heat (20 mins)
 Boiling heat (10 mins)
 Low heat (15 mins)



50 CFU/mL

Alternative Ch. method

Cooking:
 Boiling heat (25 mins)
 Low heat (60 mins)



No Growth

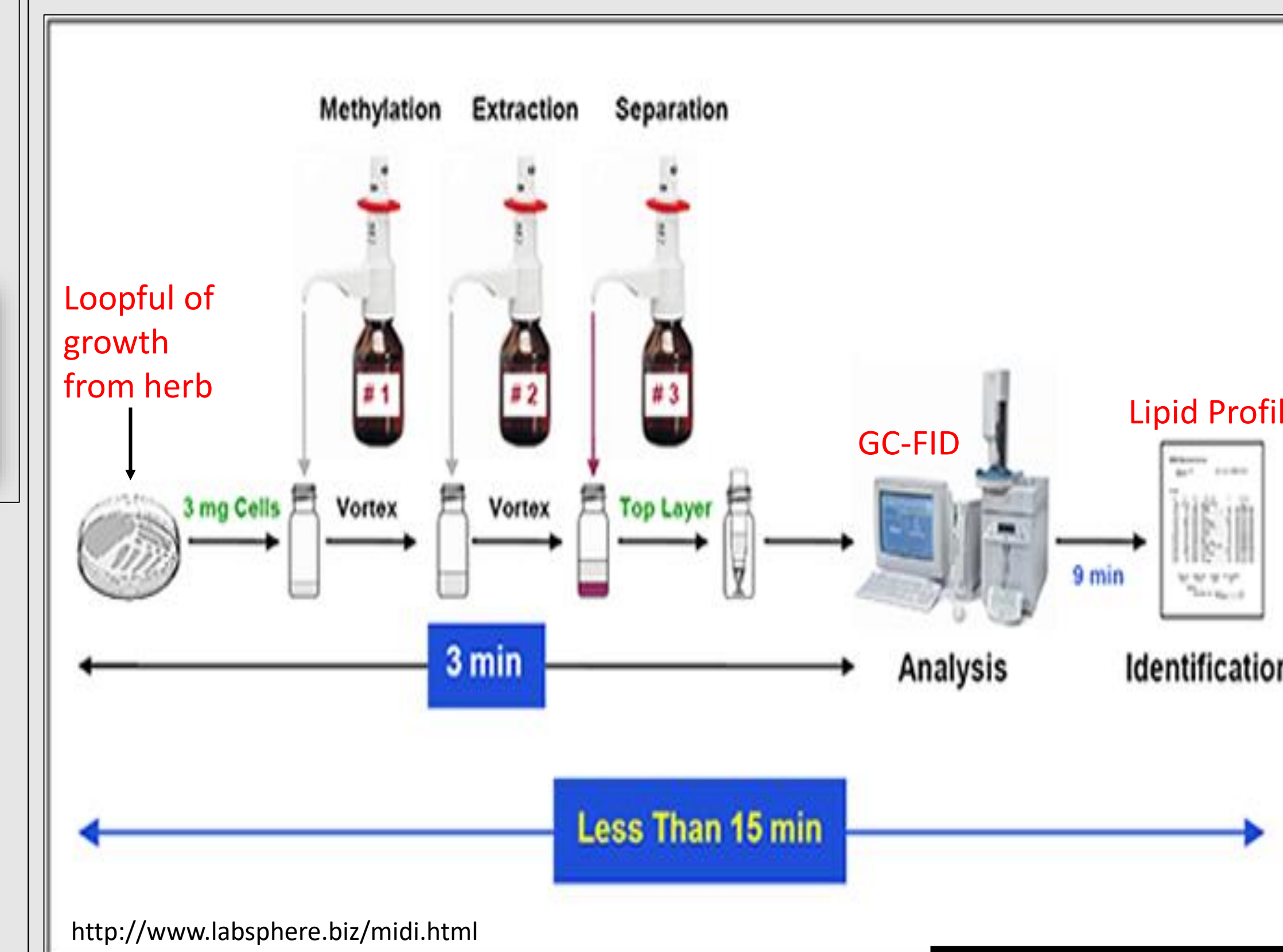
Traditional American method

Cooking:
 Steeped in boiling water (3 mins)

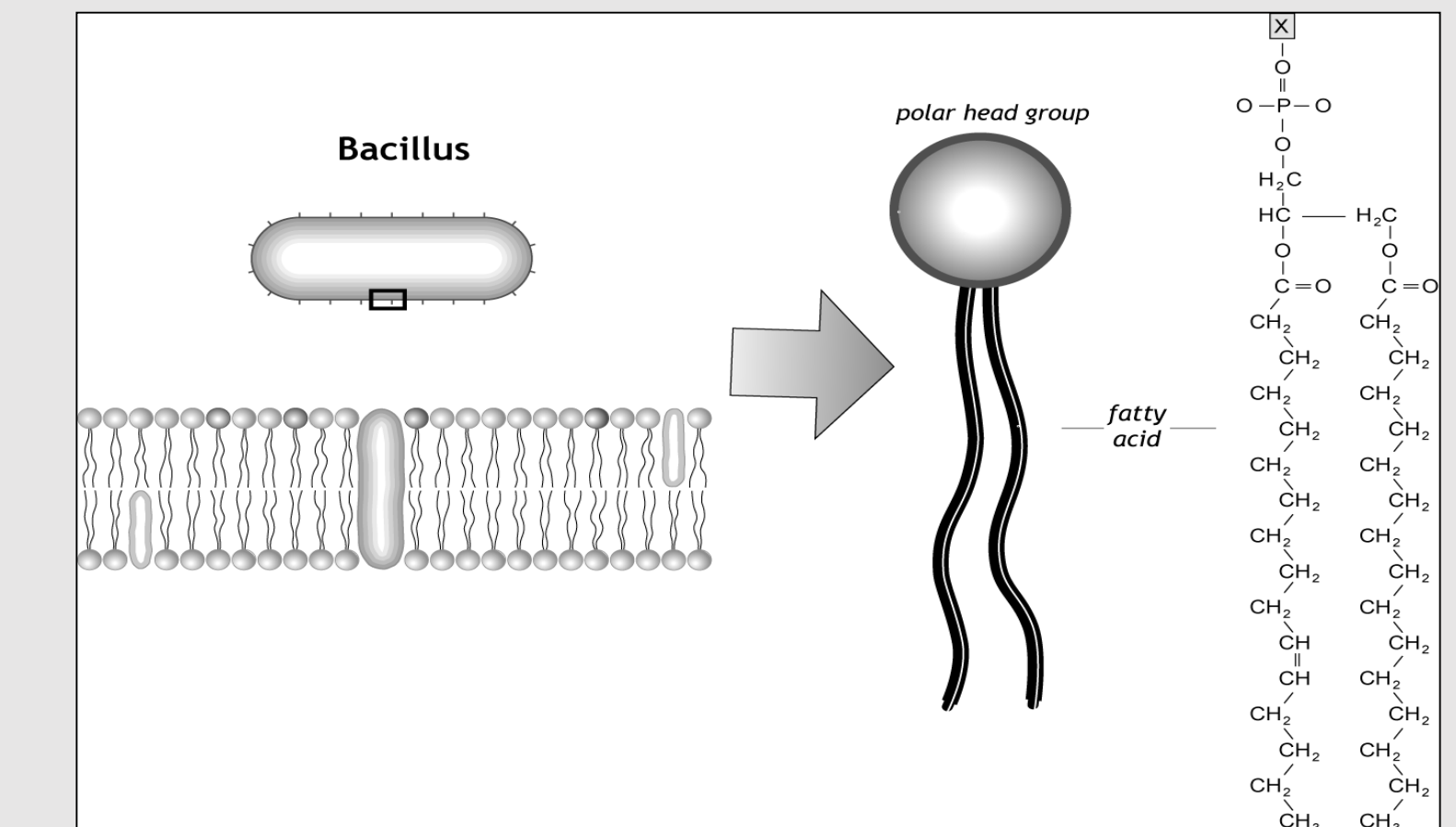


1500-3000 CFU/mL

FAME Method

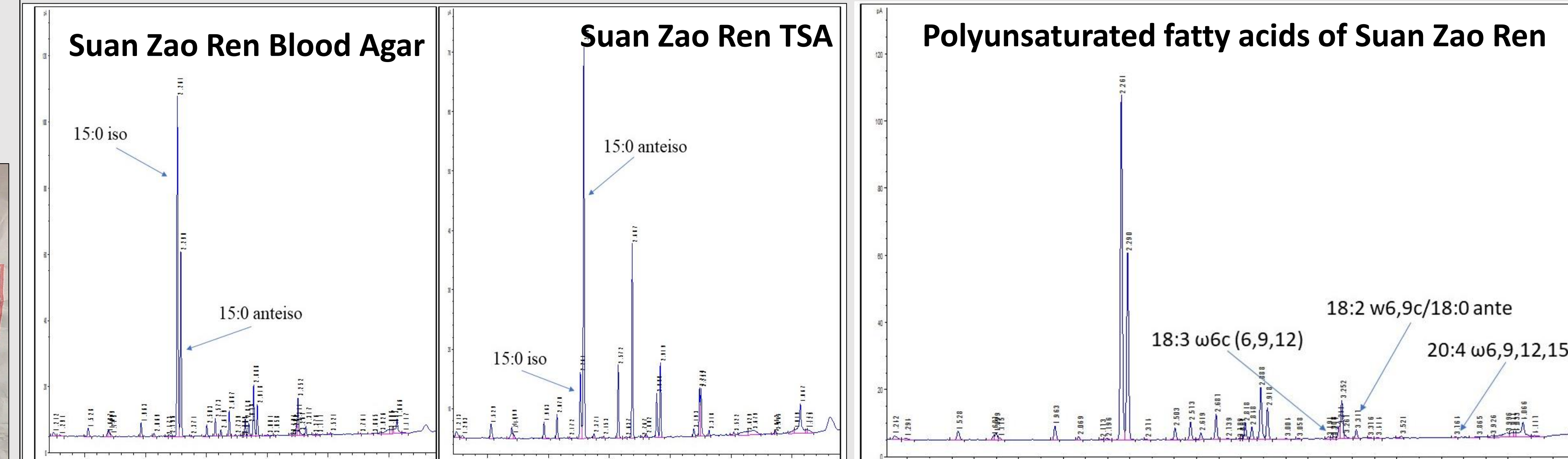


- Bacteria species have specific lipid profiles



- Herbs cultured on both Blood Agar and Tryptic Soy Agar plates
- Rapid liquid-liquid extraction
- Analysis/identification by GC-FID

Results



Herb	Ratio of 15 carbon branched chain fatty acids	<i>Bacillus</i> sp. present
Sha Yuan Zi	15:0 iso > 15:0 anteiso	<i>Bacillus</i> ACT
Chuan Duan	15:0 iso < 15:0 anteiso	<i>Bacillus</i> SM
Gou Teng	15:0 iso < 15:0 anteiso	<i>Bacillus</i> SM
Di Long	15:0 iso > 15:0 anteiso	<i>Bacillus</i> ACT
Jiang Can	15:0 iso > 15:0 anteiso	<i>Bacillus</i> ACT <i>Bacillus</i> SM
Fu Ling	15:0 iso > 15:0 anteiso	<i>Bacillus</i> ACT
Mo Yao	15:0 iso > 15:0 anteiso	<i>Bacillus</i> ACT
Suan Zao Ren	15:0 iso > 15:0 anteiso 15:0 iso < 15:0 anteiso	<i>Bacillus</i> ACT <i>Bacillus</i> SM
Tian Ma	15:0 iso < 15:0 anteiso	<i>Bacillus</i> SM
Wu Yao	15:0 iso < 15:0 anteiso	<i>Bacillus</i> SM
Yan Hu Suo	15:0 iso < 15:0 anteiso	<i>Bacillus</i> SM

- FAMES used as fatty acid biomarkers
- Presence of branched chained 15 carbon fatty acid indicates *Bacillus*
- Ratio of 15:0 iso/anteiso determines *Bacillus* species
- Polyunsaturated fatty acids are biomarkers of fungal species
 - Found in 82% of samples

Conclusions

- Established a rapid taxonomic survey of biological and fungal contaminants present on suspect herbal samples based on fatty acid biomarkers.
- The survival study demonstrated the potential for pathogenic spores to survive the brewing process.
- The bacterial and fungal identification can help to reconstruct toxicological episodes that result in medical emergencies or death.

Funding

UROF
 2017 Summer Fellowship
 NIH
 National Institute of Health
 P30DA033934

The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.