

INTRODUCTION

Foodborne diseases due to bacterial pathogens is a persistent issue and a significant safety concern to public health. Various food preservation techniques including use of chemical preservatives are adopted by food industries to help mitigate the risk of microbial growth and improve the shelf-life. However, there is a considerable concern regarding the risk of using synthetic additives on human health and consumers are demanding for more natural alternatives. Plant based antimicrobial compounds can be used as an effective natural preservatives. The antimicrobial activity of several common plant species has been widely researched but thousands of species are still unexplored for their potential antimicrobial and antioxidant properties.

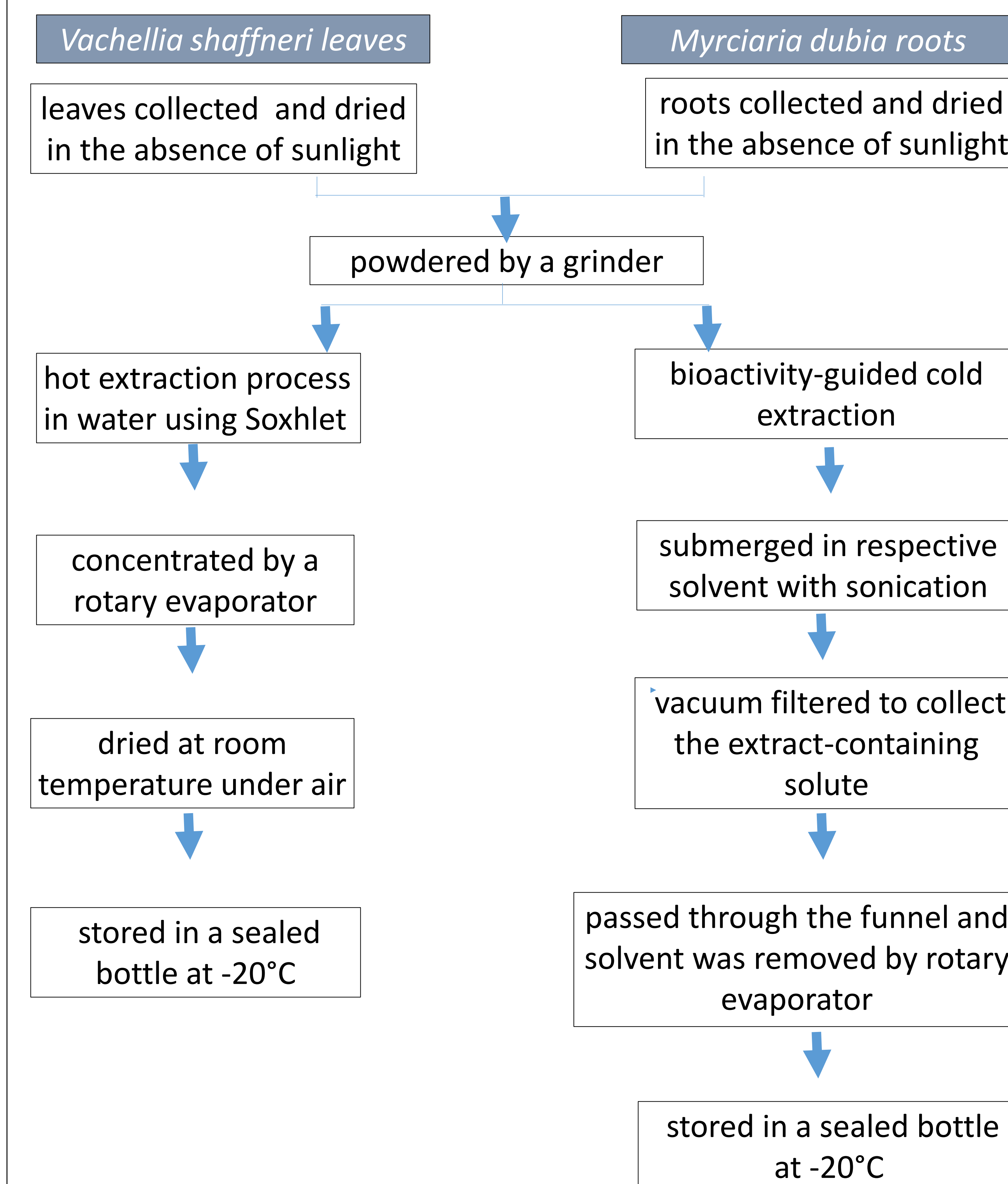
OBJECTIVES

The objectives of this study was to determine:

- (1) the antimicrobial properties of two plant species essential extracts : (i) leaves of *Vachellia shaffneri* and (ii) roots of *Myrciaria dubia* against most problematic foodborne pathogens such as *Listeria*, *Salmonella typhimurium* and pathogenic *E. coli*
- (2) Effect of different extraction methods on antimicrobial properties

METHODOLOGY

(i) Preparation of plant essential extracts

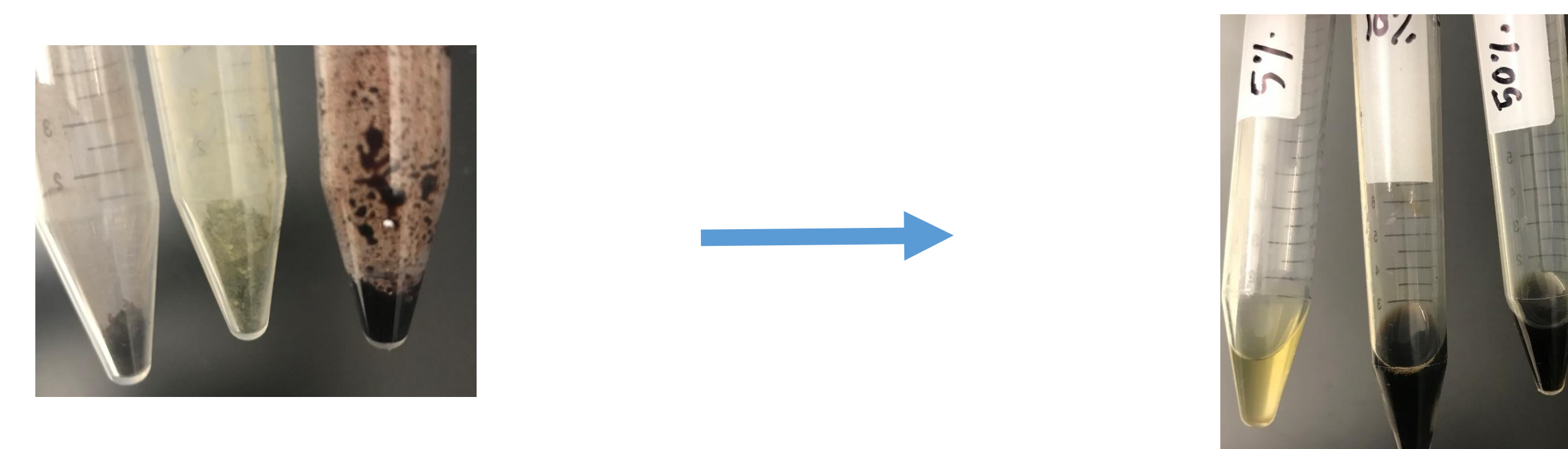


(ii) Selection of bacterial strains and inoculum preparation



- *L. innocua*,
 - *S. Typhimurium* (ATCC 14028)
 - *E. coli* O157: H7 (ATCC 35150)
- Centrifuged at 4000 rpm for 10 min, washed in 0.1% BPW and serial diluted to 10^{5-6} CFU/mL
- Enumerated after incubation at 37°C for 16–24 hrs

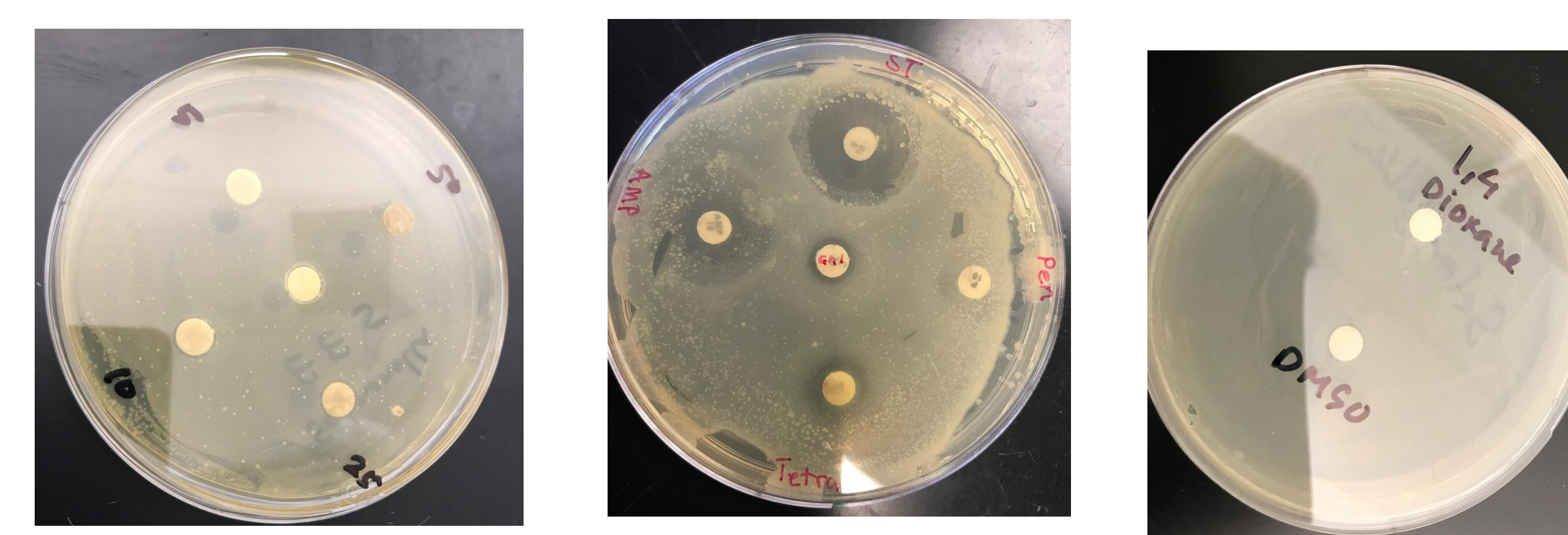
(iii) Preparation of essential extract solutions for testing



All the essential extracts were dissolved into aqueous 5% DMSO or 1,4-dioxane

5, 10, 25 and 50% concentrations of each extracts were produced

(iv) Antimicrobial activity testing by disc diffusion assay



- filter discs were soaked into the desired concentration of essential extracts
- Placed on the TSA plates with bacterial inoculum
- incubated at 37°C for 7 days and the diameter of the zone of inhibition was measured
- Tetracycline antibiotic discs (20 mg/disc) were used as positive control
- 1,4 dioxane or 5% DMSO solutions were used a negative control

RESULTS

Table 1: The mass of each extract obtained and percent by mass relative to the original weight of the *Myrciaria dubia* dry roots

Solvent	Dielectric Constant (ϵ)	Roots (grams)
Hexanes	1.9	3.06
Dichloromethane	8.9	7.23
Methanol	33.1	103.1
Water	80.1	25.47

Table 2: Antimicrobial activity by different concentration of the *Vachellia shaffneri* leaves essential extracts against foodborne pathogens

Type of pathogens	Zone of inhibition (mm)			
	5% Mean \pm SD	10% Mean \pm SD	25% Mean \pm SD	50% Mean \pm SD
<i>Listeria</i>	0.2 \pm 0.35	0.4 \pm 0.38	0.5 \pm 0.44	0.6 \pm 0.52
<i>Salmonella</i>	0.4 \pm 0.38	0.4 \pm 0.35	0.4 \pm 0.35	0.7 \pm 0.06
<i>E. Coli</i> O157:H7	0.2 \pm 0.35	0.2 \pm 0.35	0.4 \pm 0.35	0.7 \pm 0.06

Table 3: Antimicrobial activity by different concentration of the various types of *Myrciaria dubia* roots essential extracts against foodborne pathogens

Type of extract	Type of pathogens	Zone of inhibition			
		5% Mean \pm SD	10% Mean \pm SD	25% Mean \pm SD	50% Mean \pm SD
Water	<i>Listeria</i>	0.2 \pm 0.35	0.2 \pm 0.35	0.5 \pm 0.42	0.4 \pm 0.38
	<i>Salmonella</i>	0.4 \pm 0.35	0 \pm 0	0.4 \pm 0.38	0.6 \pm 0
	<i>E. Coli</i> O157:H7	0.2 \pm 0.35	0.2 \pm 0.35	0.6 \pm 0.06	0.6 \pm 0
Methanol	<i>Listeria</i>	0.2 \pm 0.35	0.2 \pm 0.35	0.5 \pm 0.46	0.5 \pm 0.44
	<i>Salmonella</i>	0.3 \pm 0.52	0.3 \pm 0.46	0.7 \pm 0.17	0.8 \pm 0.12
	<i>E. Coli</i> O157:H7	0.3 \pm 0.52	0.3 \pm 0.52	0.7 \pm 0.17	0.8 \pm 0.12
Dichloromethane (DCM)	<i>Listeria</i>	0.6 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.0	0.7 \pm 0.1
	<i>Salmonella</i>	0.6 \pm 0.0	0.6 \pm 0.0	0.8 \pm 0.1	0.6 \pm 0.0
	<i>E. Coli</i> O157:H7	0.7 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.1
Hexanes	<i>Listeria</i>	0.6 \pm 0.1	0.6 \pm 0.1	0.6 \pm 0.0	0.7 \pm 0.1
	<i>Salmonella</i>	0.7 \pm 0.1	0.6 \pm 0.1	0.6 \pm 0.1	0.7 \pm 0.1
	<i>E. Coli</i> O157:H7	0.6 \pm 0.0	0.6 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.1

SUMMARY

- *Salmonella* was found to be more susceptible than *Listeria* and *E. coli* against the essential extract from *Vachellia shaffneri* leaves
- DCM and hexane extract from *Myrciaria dubia* roots showed higher inhibition compared to other three extracts ($P < 0.5$).
- No significant difference in the inhibition was observed among the three bacterial strains upon exposure to different types of *Myrciaria dubia* extracts .
- 25 and 50% concentrations of all essential extracts showed more inhibition than 5 or 10%
- Tested essential oil extracts showed promise to use natural antimicrobial compounds. However, further studies required to better understand mechanism of antimicrobial activities.

ACKNOWLEDGEMENTS

This work was supported by the UTRGV startup funds, and UT System Rising STARs Award to Dr. Yemmireddy. We acknowledge Mr. Edgar Vasquez for collecting the *Vachellia shaffneri* leaves and Jorge Flores for collecting the *Myrciaria dubia* roots from the Amazon (Peruvian) rainforest. A special thanks to Mr. Flores for conducting the polarity-based bioactivity-guided extraction of the *Myrciaria dubia* roots.