Antibacterial Activity of *Ilex paraguariensis* (Yerba Mate) Against Gram Positive and Gram Negative Bacteria.

http://skinnywithfiber.org

Roula M. Abdel-Massih

Dept. of Biology, University of Balamand, Lebanon

Ilex paraguariensis

Classification and characteristics

- Family Aquifoliaceae
- Found mainly in South America
- Herbal tea beverage
- Dry leaves (50g) are packed in gourd + hot water poured over them (1L)

http://skinnywithfiber.org

http://skinnywithfiber.org

Ilex paraguariensis

Active Phytochemicals

Active phytochemicals

Polyphenols:
chlorogenic acid
xanthines : (caffeine, theobromine)

Flavonoids :
quercetin, kaempferol, rutin

Purine alkaloids
caffeic acid;
3,4-dicaffeoylquinic
3,5-dicaffeoylquinic acid

Vitamins (C, B1, B2)

Minerals
(P, Fe, Ca);

a.a.

Ilex paraguariensis

Active Phytochemicals: Polyphenols

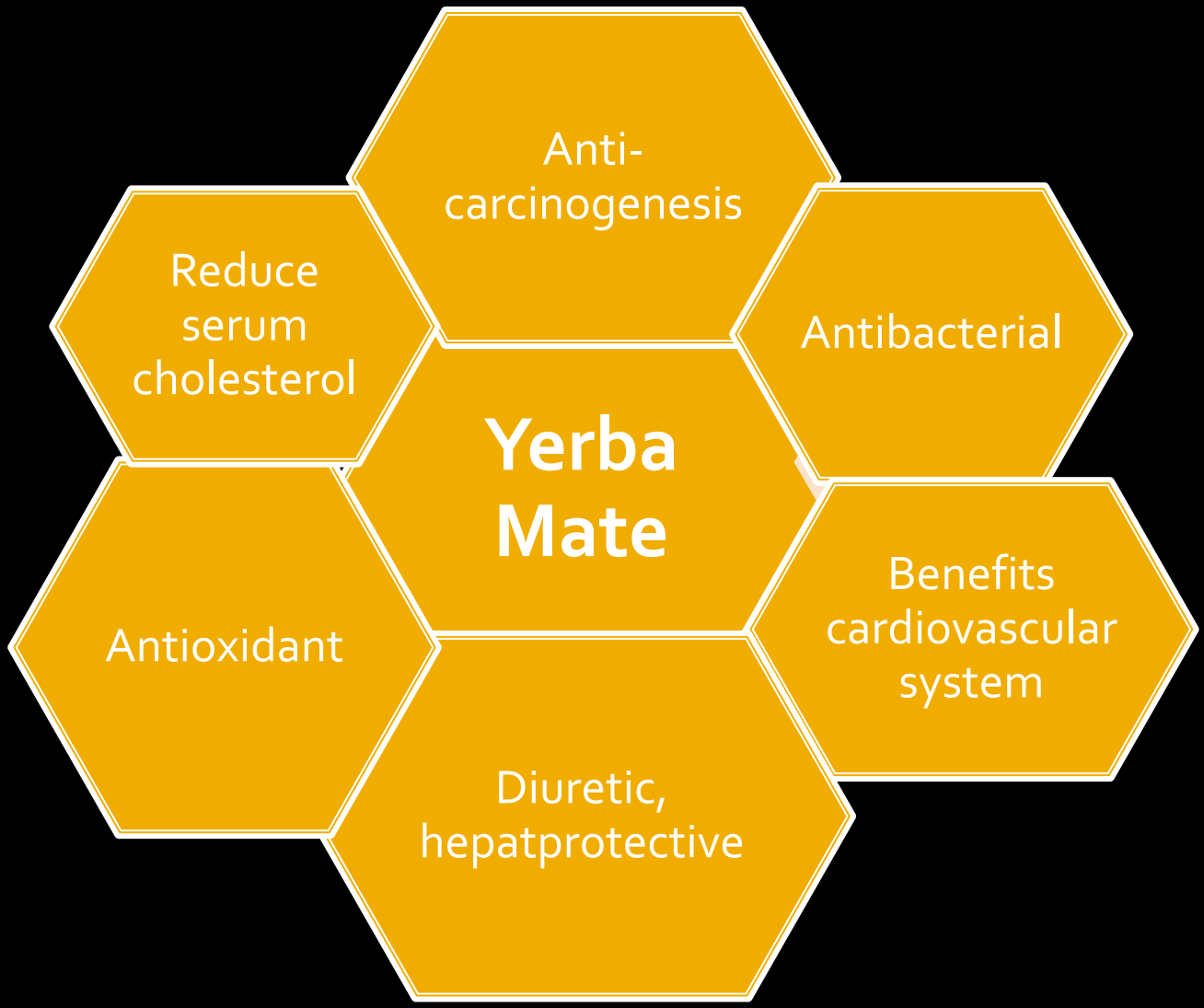
Table 1—Polyphenols in green tea, black tea, and Mate tea.[a]

	Green tea	Black tea	Mate tea
Caffeic acid	•		•
Caffeine	•	•	•
Caffeoyl derivatives			•
Caffeoylshikimic acid			•
Catechin	•	•	
Catechin gallate		•	
Chlorogenic acid			•
Coumaric acid	•		
Epicatechin gallate	•	•	
Epigallocatechin	•		
Epigallocatechin gallate	•		
Feruloylquinic acid			•
Gallic acid	•	•	
Gallocatechin gallate	•	•	
Kaempferol	•	•	•
Myricetin	•	•	
Procyanidin		•	
Quercetin	•	•	•
Quinic acid	•		•
Rutin	•	•	•
Theaflavin		•	
Theobromine		•	•

[a]Adapted from Carini and others (1998); Chandra and de Mejia Gonzalez (2004); Atoui and others (2005); Bastos and others (2007); Bravo and others (2007).

Ilex paraguariensis

Properties



Objectives

- To determine the antibacterial activity of the **aqueous extract** of Mate against selected Gram positive and Gram negative bacteria.
- To study the potential synergistic/ antagonistic effect of Mate combined with different classes of antibiotics with different mechanisms of action
- To elucidate the interactions between the intake of Mate and an antibiotic during a course of treatment

Ilex paraguariensis Extraction

- Dry Mate leaves (Amanda) O/N
- Blend with food blender→ add sterile deionized water (3.6ml/1g ground mate) → heat to 70° C for 2h

- Then filter with Whatman filter paper
- Centrifuge at 5000 g for 30min → conc SN at 70° C → dialyze → Dry using speedvac

- Dissolve dried extract in water at 120mg/ml
- Store at 4°C

Ilex paraguariensis

Method

Mate extract

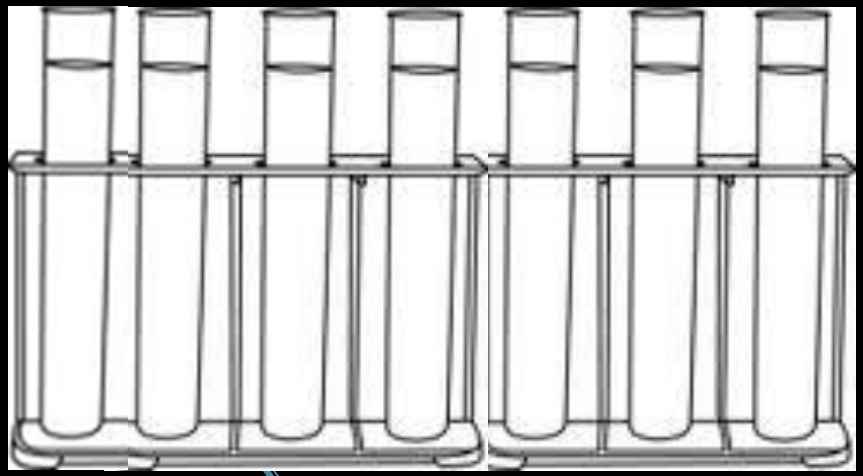
(50 µL of 120mg/ml) +

Mueller Hinton

Broth (150 µl) :

15mg/ml $conc_f$ in tube 1

100 µL of bacterial suspension 10^6 CFU



Serial Dilution

MIC

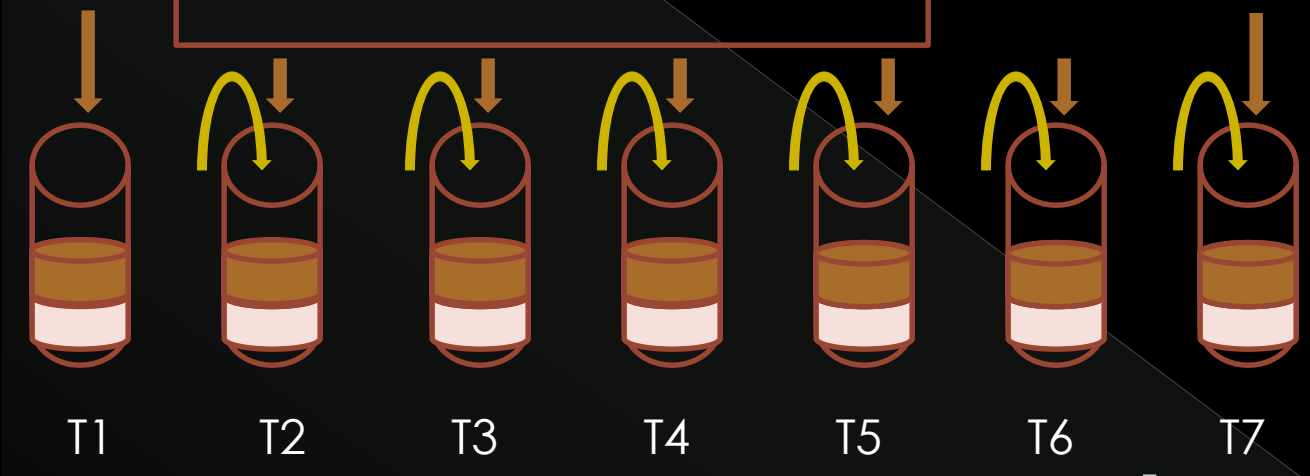
MBC

MIC determination

c- To T1, add 4 times the required highest concentration after removing the same volume to be added

d- Mix well

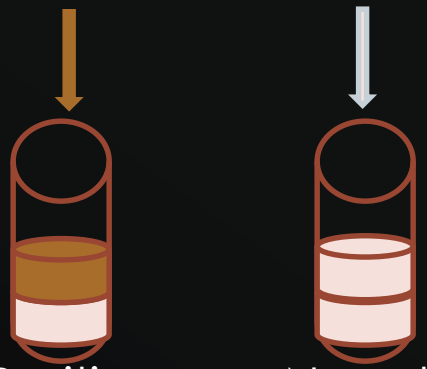
e- Transfer 1 ml from T1 to T2
Mix well, and transfer from T2 to T3
and continue to T7, discard the last 1 ml



T1 T2 T3 T4 T5 T6 T7

f- Add 1 ml 10^6 cfu/ml to all tubes except Negative Control

a- Add 1 ml of sterile MHB
b- 2 ml of sterile MHB to T1

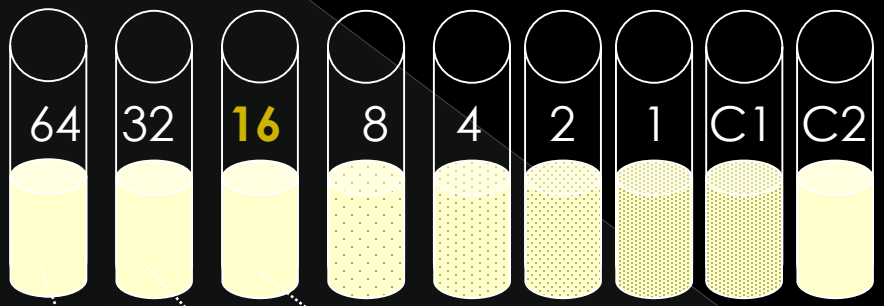


Positive Control

Negative Control

g- Add 1 ml MHB to the negative control tube

MIC and MBC



0.01 ml (spread plate), Incubate 35 °C, o/N

Day 2

Record visual turbidity

Subculture non-turbid tubes to agar plates

MIC = 16 mg/l

Day 3

MBC = 32 mg/l

Table 1 (a): Antibacterial activity of aqueous extract of *Ilex paraguariensis*

	Bacteria Strains	MIC (mg/ml)	MBC (mg/ml)
ATCC strains	*E. coli* ATCC 25922	1.875	3.75
	S. aureus ATCC 29213	0.468	0.468
	Pseudomonas aeroginosa ATCC 27853	1.875	1.875
	Acinetobacter baumanii ATCC 17978	0.468	0.468

A greater antibacterial activity was observed against reference *S. aureus* and *Acinetobacter baumanii* with MIC & MBC of 0.468 mg/ml.

Table 1 (b): Antibacterial activity of aqueous extract of *Ilex paraguariensis*

	Bacteria Strains	MIC (mg/ml)	MBC (mg/ml)
Wild (S)	*E. coli* 1	7.5	7.5
	E. coli 2	7.5	15
ESBL +	*E. coli* ESBL 1	1.875	3.75
	E. coli ESBL 2	7.5	7.5
	E. coli ESBL 3	7.5	15
	E. coli ESBL 4	3.75	15
AmpC +	*E. coli* AmpC	7.5	15
	E. coli AmpC	3.75	3.75
ESBL+ AmpC	*E. coli* ESBL + AmpC 1	3.75	7.5
	E. coli ESBL + AmpC 2	15	15
Oxa 48	*E. coli* OXA48 (carba resistant)	3.75	3.75

Table 1 (c): Antibacterial activity of aqueous extract of *Ilex paraguariensis*

	Bacteria Strains	MIC (mg/ml)	MBC (mg/ml)
Wild (S)	*Klebsiella pneumonia* 1	7.5	15
	Klebsiella pneumonia 2	7.5	15
ESBL +	*Klebsiella pneumonia* ESBL 1	1.875	1.875
	Klebsiella pneumonia ESBL 2	15	15
	Klebsiella pneumonia ESBL 3	15	15
Oxa-48	*Klebsiella pneumonia* Oxa-48 (carba resistant)	3.75	3.75
Wild type Enterics	*Serratia marcesens* 1	7.5	7.5
	Enterobacter aerogenes 1	3.75	7.5
	Enterobacter agglomerans 1	7.5	7.5
	Proteus mirabilis 1	3.75	7.5

Table 1 (d): Antibacterial activity of aqueous extract of *Ilex paraguariensis*

	Bacteria Strains	MIC (mg/ml)	MBC (mg/ml)
Wild (S)	*Enterococcus faecalis* 1	1.875	1.875
	Enterococcus faecalis 2	0.468	1.875
Wild (S)	*S. aureus* MSSA 1	1.875	3.750
	S. aureus MRSA 2	1.875	1.875

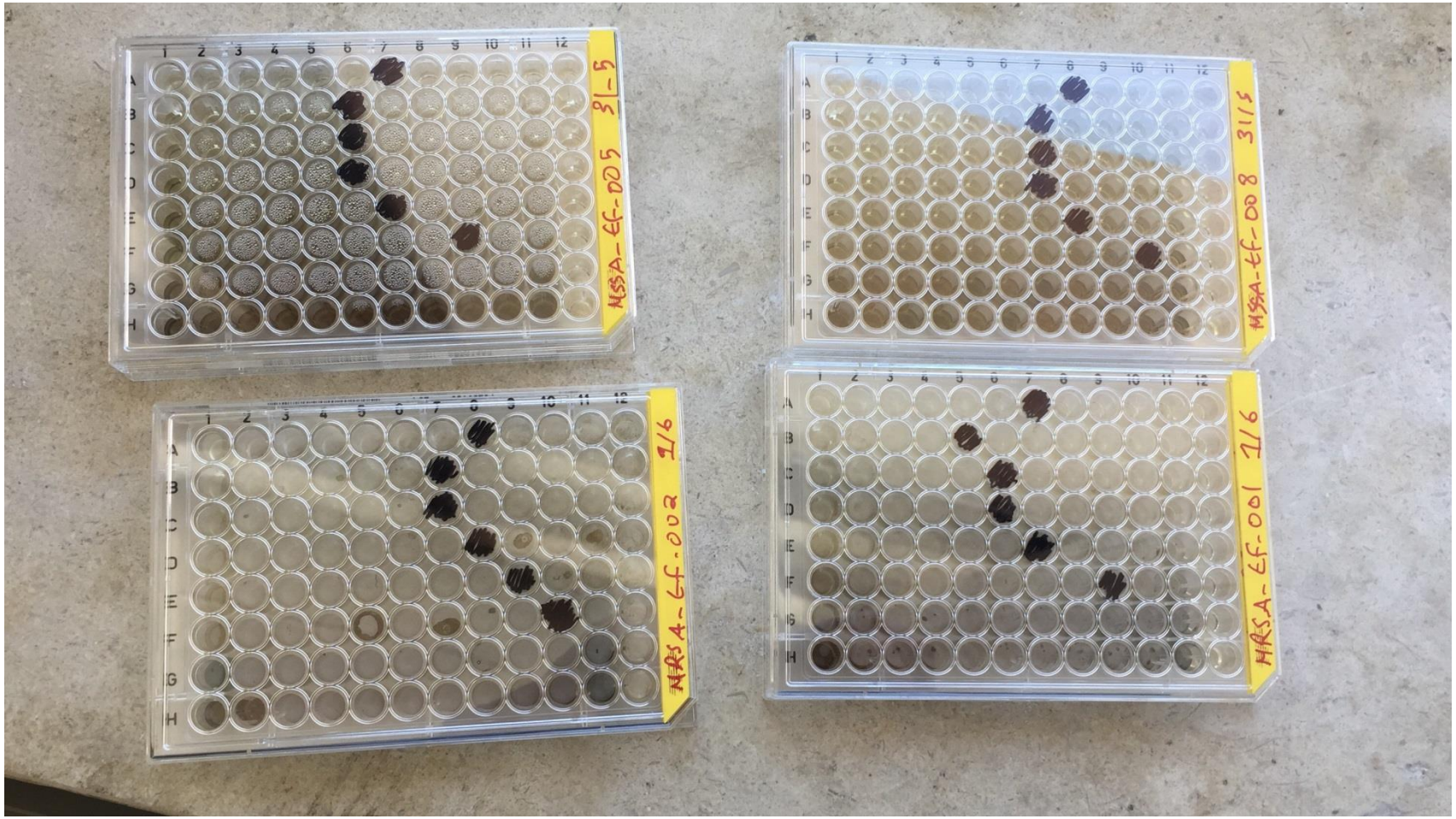
Objectives

- To determine the antibacterial activity of the **aqueous extract** of Mate against selected Gram positive and Gram negative bacteria.
- To study the potential synergistic/ antagonistic effect of Mate combined with different classes of antibiotics with different mechanisms of action
- To elucidate the interactions between the intake of Mate and an antibiotic during a course of treatment

Combination essays
Mate/ Gentamycin

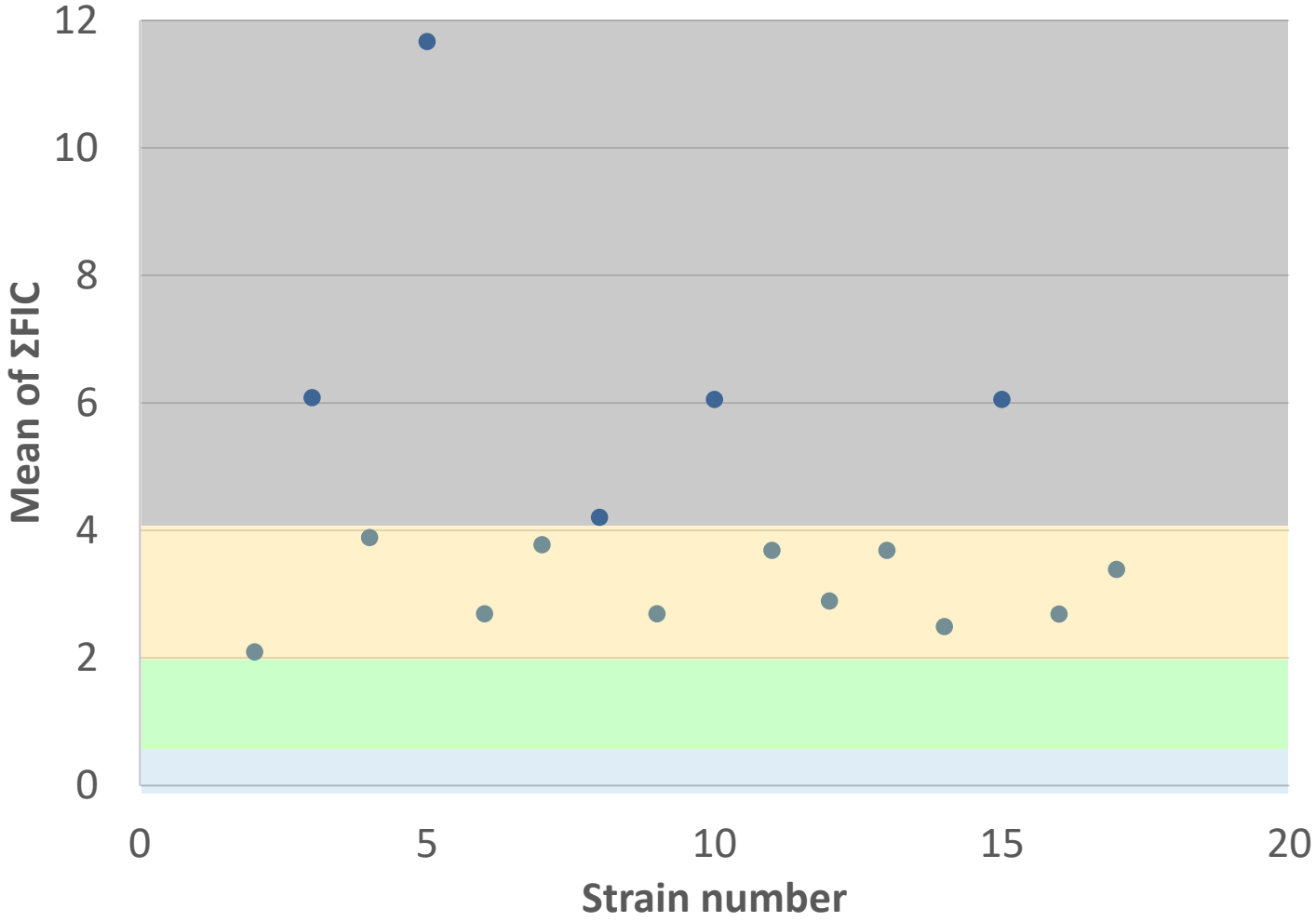
Genta	1	2	3	4	5	6	7	8	9	10	11	12
A		0.000977	0.001953	0.003906	0.007813	0.015625	0.03125	0.0625	0.125	0.25	0.5	1
B	0.3125	0.000977	0.001953	0.003906	0.007813	0.015625	0.03125	0.0625	0.125	0.25	0.5	1
C	0.625	0.001309	0.002617	0.005234	0.010469	0.020938	0.041875	0.08375	0.1675	0.335	0.67	1
D	1.25	0.001309	0.002617	0.005234	0.010469	0.020938	0.041875	0.08375	0.1675	0.335	0.67	1
E	2.5	0.001309	0.002617	0.005234	0.010469	0.020938	0.041875	0.08375	0.1675	0.335	0.67	1
F	5	0.001309	0.002617	0.005234	0.010469	0.020938	0.041875	0.08375	0.1675	0.335	0.67	1
G	10	0.001309	0.002617	0.005234	0.010469	0.020938	0.041875	0.08375	0.1675	0.335	0.67	1
H	10	0.001309	0.002617	0.005234	0.010469	0.020938	0.041875	0.08375	0.1675	0.335	0.67	NC

Some of the Checkerboards that were done consisting of Gentamicin and Mate; colored wells represent the different FICs



Mean ΣFIC for Gentamicin & Mate for *S. aureus* strains

No synergy, No additive, 11 indifferent, 5 antagonistic



FICs between
2 <x< 12

Mate was mostly Antagonistic to Gentamicin activity

Combination essays
Mate/ Cefotaxime

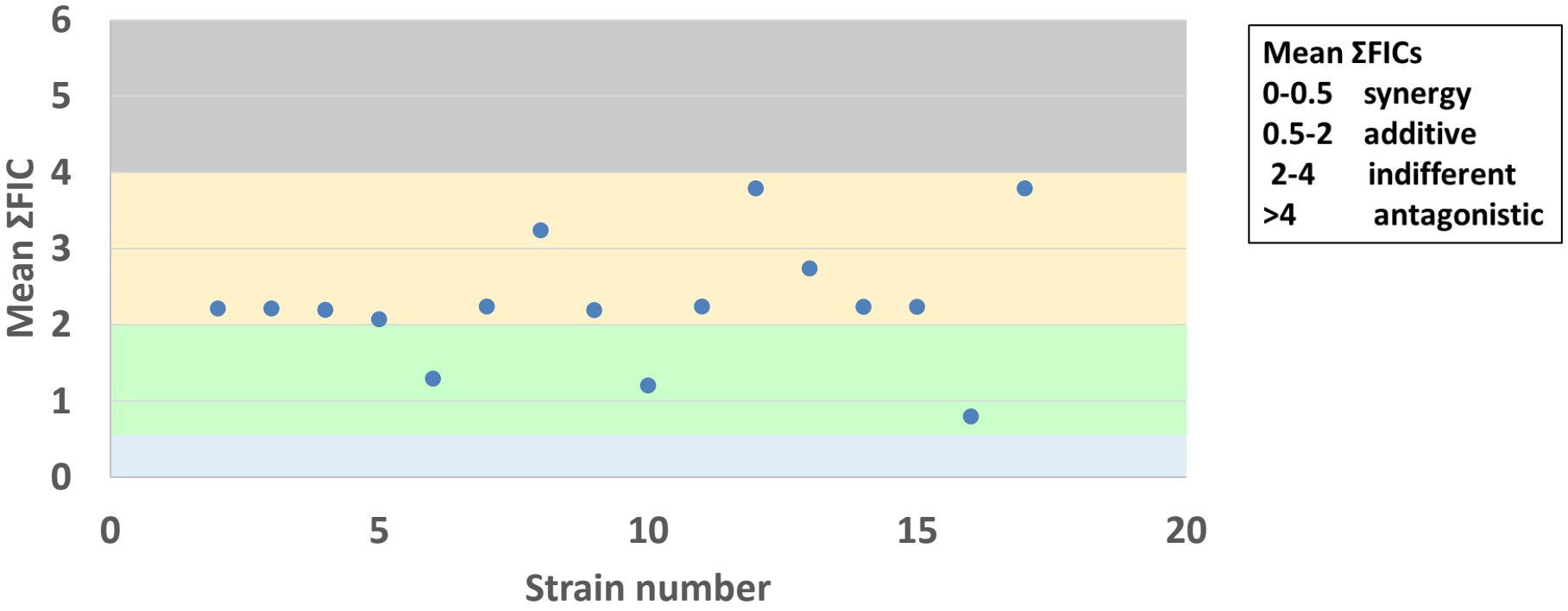
CTX	1	2	3	4	5	6	7	8	9	10	11	12
A		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	32
B		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	32
C		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	32
D		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	32
E		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	32
F		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	32
G		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	32
H		0.041602	0.083203	0.166406	0.332813	0.665625	1.33125	2.6625	5.325	10.65	21.3	NC

Mate	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125	
C	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	
D	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	
E	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	
F	5	5	5	5	5	5	5	5	5	5	5	
G	10	10	10	10	10	10	10	10	10	10	10	
H	10	10	10	10	10	10	10	10	10	10	10	NC

The cells highlighted in yellow represent the various FICs whereas the cells highlighted in red represent the MIC of each compound alone

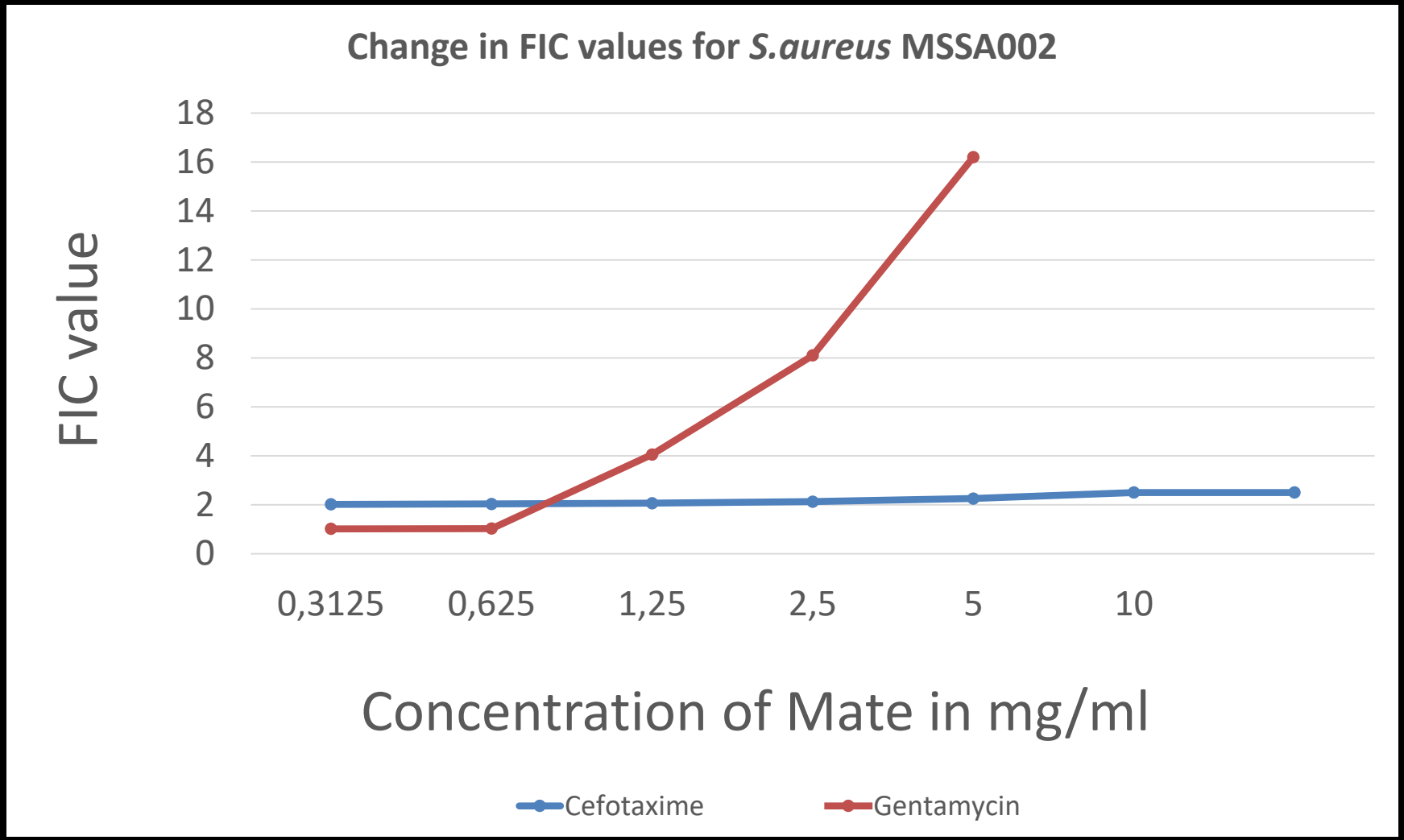
Mean ΣFIC for Cefotaxime & Mate for *S. aureus* strains

No synergy, 3 additive, 13 indifferent, no antagonistic



Mate was mostly indifferent to Cefotaxime activity

FIC of Cefotaxime & Gentamycin with increasing Mate concentration



Ilex paraguariensis
Conclusion

- The antibacterial activity of the extract did not show any correlation with the profile of resistance of the tested bacteria.
- In general, the MIC and MBC values ranged between 0.468 mg/ml and 15 mg/ml.
- **A greater antibacterial activity was observed against reference *S. aureus* and *Acinetobacter baumanii* with MIC & MBC of 0.468 mg/ml.**

Ilex paraguariensis

Conclusion

- For each antibiotic, 16 different *S. aureus* strains were tested and all conformed to the same pattern showed in the graph
- **Indifference with Cefotaxime**
- **Antagonism with Gentamicin in a concentration dependent way**

Ilex paraguariensis
Conclusion

- A more in-depth analysis is needed to
  - identify the active molecule responsible for this activity
  - test a wider range of bacterial isolates for a better understanding of the potential role of Yerba Mate in developing new antibacterial agents
  - elucidating Mate's interactions with antimicrobial agents used in the clinical setting.

Thank You

University of Balamand, Lebanon