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SYNERGISTIC EFFECTS OF ANTIBIOTICS IN COMBINATION WITH ESSENTIAL OILS AGAINST PATHOGENIC BACTERIAL STRAINS

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INTRODUCTION

Infectious disease is a major health concern worldwide.

Antibiotics were developed.

Drug-resistant bacteria threatens successful treatment.

Combination therapy as an alternative to modify bacterial resistance.

Research Problems

LITERATURE REVIEW

Sharp, aromatic scent

Volatile

Essential oils (Koul *et al.*, 2008)

Antimicrobial properties

In fluid droplets, stem, leaves, barks, fruits or roots

SYNERGISM

A phenomenon in which the combination of two drugs results in a **greater effect** than the sum of either one given separately.

If the combination results in a **declining effect** less to the sum of either antibiotic given alone, it is called **antagonism.**

Indifference or additive effect occurs when two drugs combined has no effect and produce equal sum as given separately

Zingiberaceae

- Genus: Zingiber
- Dispersed throughout tropical Australia and East Asia.
- Used as spices and flavouring in Southeast Asia (Yob *et al.*, 2011)
- ‘Zingiber’ means bull’s horn in Sanskrit (Larsen *et al.*, 1999)

Curcuma mangga Val.

- **Voucher specimen no:** PIIUM 0207
- **Common name**: 'Temu pauh', 'kunyit mangga' (Malek *et al.*, 2011)
- Smells like unripe mango when cut (Wong *et al.*, 1999)
- **Main compound**: **Caryophyllene oxide**
- Relieve stomach aches, fever, wound healing in post partum treatment (Abas *et al.*, 2005; Hong *et al.*, 2001; Park and Kim, 2002)
- **Anti-inflammatory** (Kaewkroek *et al.*, 2009; Ruangsang *et al.*, 2009)
- **Anticancer**
- **Antioxidant**
- **Antitumor** (Huang *et al.*, 1994; Abas *et al.*, 2005)
- **Anti-allergy** (Tewtrakul and Subhadhirasakul, 2007)
- **Antiprotozoal** and **antibacterial** (Habibi *et al.*, 2000)

Zingiber officinale var. rubrum Thelaide

- **Voucher specimen no:** PIIUM 0206
- **Common name**: 'halia bara'
- **Bioactive component**: **geranial**
- **Folk medicine** (*jamu*): Post partum medicine, treat rheumatic pains, tumors (Ibrahim *et al*; 2008)
- **Morphology**: more pungent and reddish (Sivasothy *et al.*, 2011)
- **Antibacterial activity**:
  (Sunilson *et al.*, 2009; Sivasothy *et al.*, 2011)

Zingiber zerumbet (L.) Smith

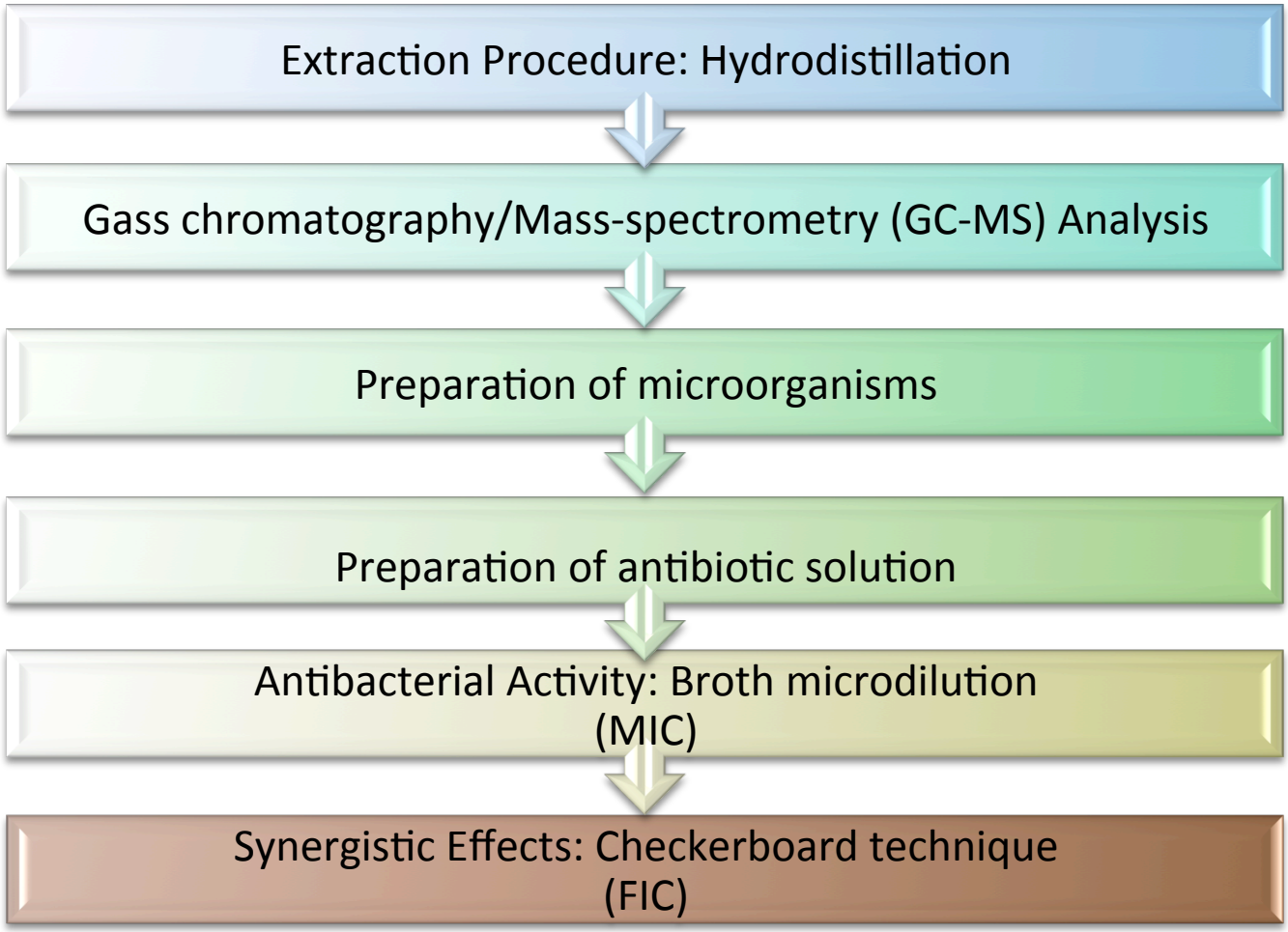
- **Voucher specimen no:** PIIUM 0208
- **Common name**: 'lempoyang' in Malay
- **Main active component**: **zerumbone** (Chien *et al.*, 2008)
- **Flavouring agents** (Nik Norulaini, 2009; Sulaiman *et al.*, 2010)
- **Antibacterial** (Kitayama, 2001)
- **Antitumor** (Murakami *et al.*, 2004)
- **Antinociceptive** (Sulaiman *et al.*, 2009)
- **Anti- inflammatory** (Zakaria *et al.*, 2011)

Objectives

To evaluate the *in vitro* interaction between 5 antibiotics and essential oils of *C. mangga*, *Z. officinale* and *Z. zerumbet* against 2 types each of Gram-positive and Gram-negative bacteria

To assist in reducing the resistance of bacteria and increase their susceptibility through synergism.

Methodology



1) Hydrodistillation

Rhizomes were purchased from a local market in Kuantan.

Washed, chopped and put into Clevenger-type apparatus.

Heated:

-8 hours

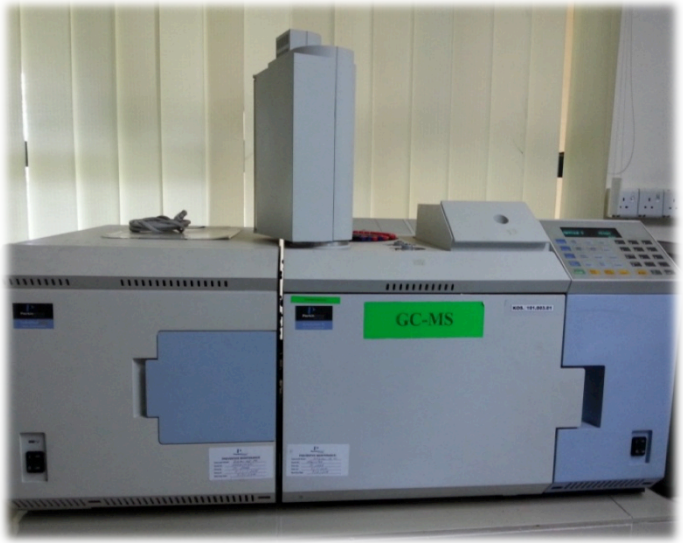
Filtrate separated using dichloromethane (DCM) in a separating funnel.

Collected oil filtered with anhydrous magnesium sulphate.

DCM separated from oil by a rotary evaporator

The obtained essential oil was kept in a universal bottle in a refrigerator and stored at 4 °C until used.

2) GC-MS Analysis



The essential oil components were identified by comparing their mass spectra (MS) fragmentation pattern and relative retention time with the National Institute of Standards and Technology (NIST) mass spectral database library
(Kamazeri *et al.*, 2012).

$$\text{Relative percentage of peak area} = \frac{\text{Area of the peak}}{\text{Total peak area}} \times 100\%$$

3) Preparation of microorganisms

Bacterial strains

- ***S. aureus*** (ATCC 25923)
- ***B* . cereus** (ATCC 11778)
- ***E. coli*** (ATCC 25922)
- ***P. aeruginosa* (**ATCC 25923)

Subculture on Petri dish

- 28 g Nutrient agar + 1 L distilled water

Culture in broth

- 8 g Nutrient broth + 1 L distilled water

Incubate

- Incubate at 37 °C for 24 hours.

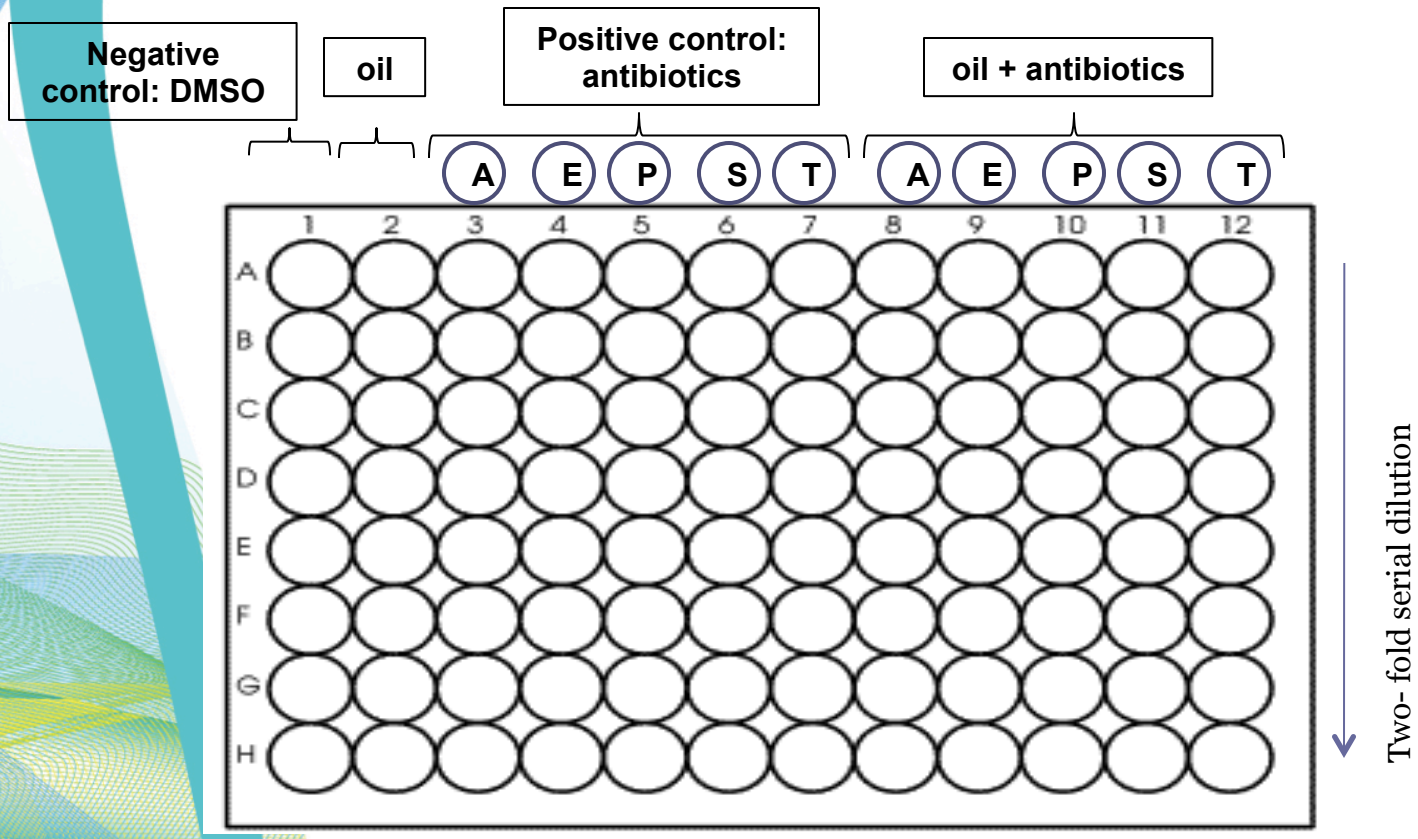
4) Preparation of antibiotic stock solution

1) Ampicillin (A)
2) Erythromycin (E)
3) Penicillin (P)
4) Streptomycin (S)
5) Tetracycline (T)

Using the formula:

$$\text{Weight of powder (mg)} = \frac{\text{Volume of solvent, DMSO (ml)} \times \text{Concentration (µg/ml)}}{\text{Potency (µg/mg)}}$$

5) Minimum Inhibitory Concentration (MIC)



Essential oil range:
10 – 0.078 µL / mL

Antibiotic range:
50 – 0.390 mg/ mL

Essential oil + antibiotics range:
60 – 0.468 µL / mL

- MIC is the lowest concentration of agents (oils and antibiotics) which inhibits visible growth of microorganisms (Okusa, 2007).
- Wells with no turbidity is taken as the MIC.

5) Fractional Inhibitory Concentration (FIC)

- FIC Index = FIC oil + FIC antibiotic
- **FIC oil**
  = MIC of oil + antibiotic in combination / MIC of oil alone
- **FIC antibiotic, A :**
  = MIC of oil + antibiotic A in combination / MIC of antibiotic A1 alone
- FIC evaluation (Mackay *et al*.,2000) :

≤ 0.5 : SYNERGY

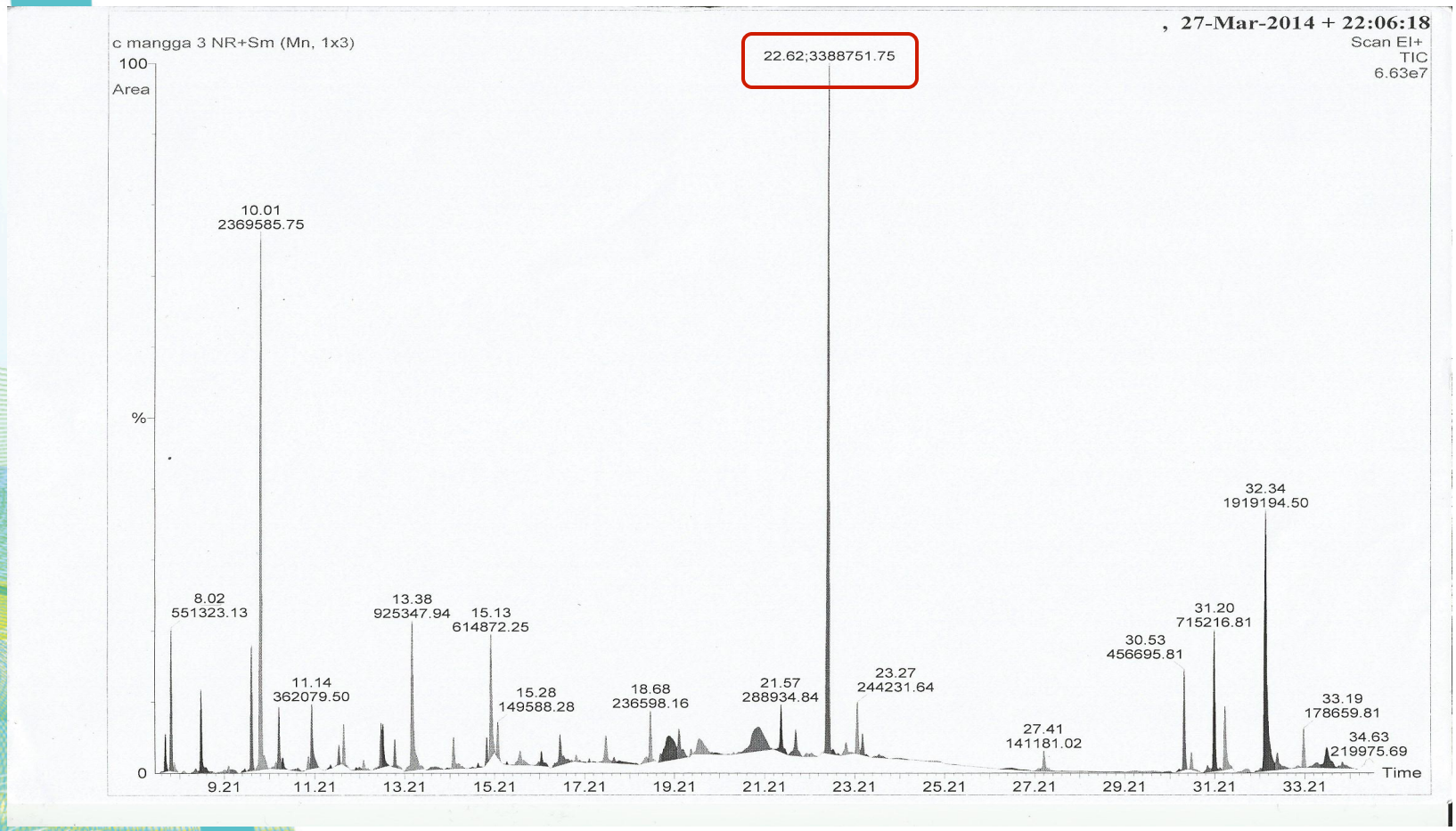
0.5 -1 : ADDITIVE

1-2 : INDIFFERENCE

> 2 : ANTAGONISM

RESULTS

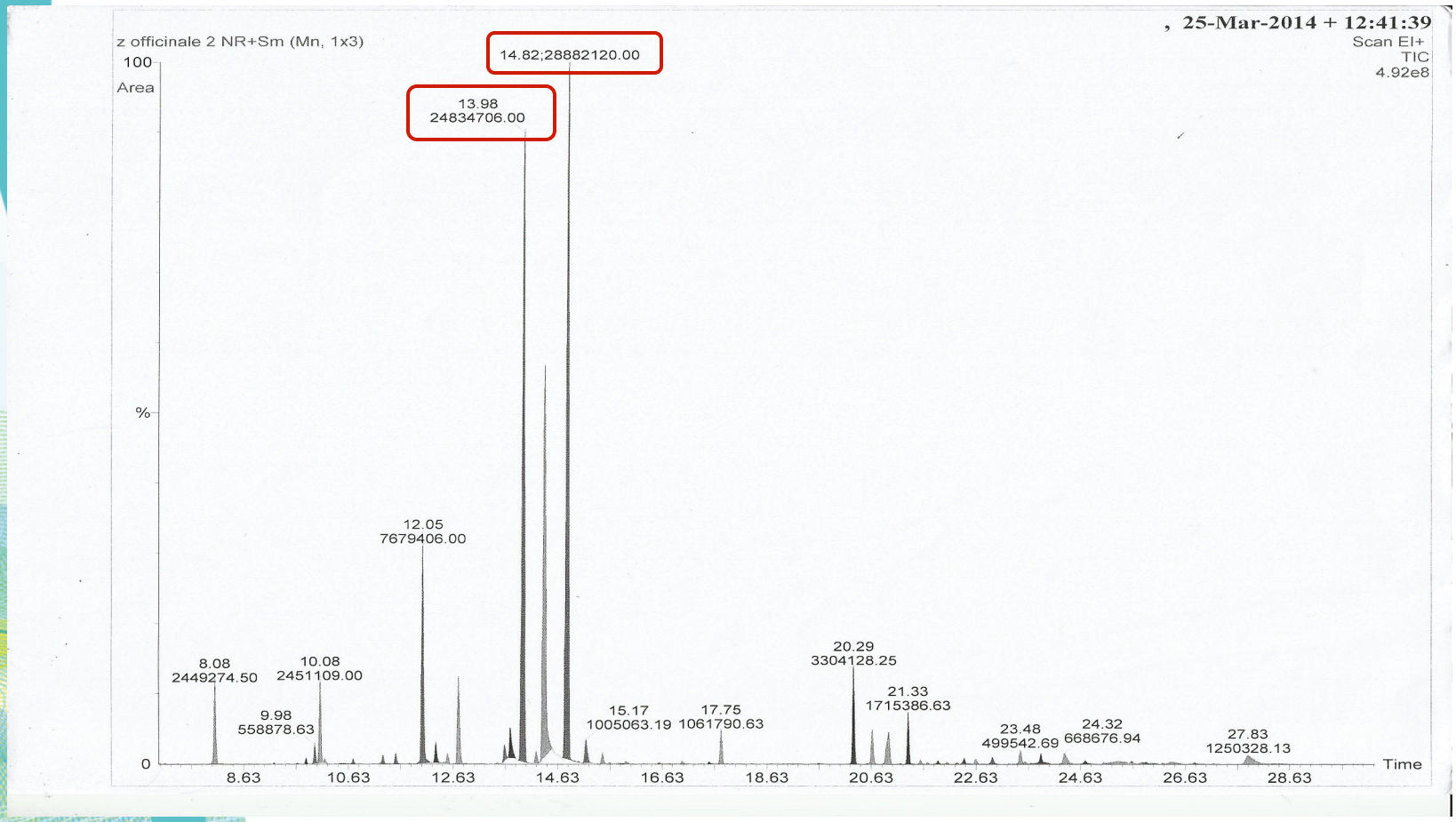
Mass spectrum of *C. mangga* essential oil



Compounds Identified

Curcuma mangga	%
Limonene	4.32
Furan, 3-(4-methyl-3-pentenyl)-	18.567
L-pinocarveol	2.837
Propanoic acid, 2-methoxy-	7.251
Cyclopropanecarboxaldehyde, 2-methyl-2-(4-methyl-3-pentenyl)-	4.818
Cyclooctanol	1.172
Caryophyllene	1.854
1, 6-Octadiene,3,5-dimethyl	2.264
Caryophyllene oxide	**26.553**
12-Oxacybicyclol[9.1.0]dodeca-3, 7-diene, 1,5,5,8-tetramethyl-	1.914
2,5-Octadiene,3,4,5,6-tetramethyl	1.10
α-farnesene	3.578
β-farnesene	6.944
Geranyl Linalool	15.058
Farnesol	1.724

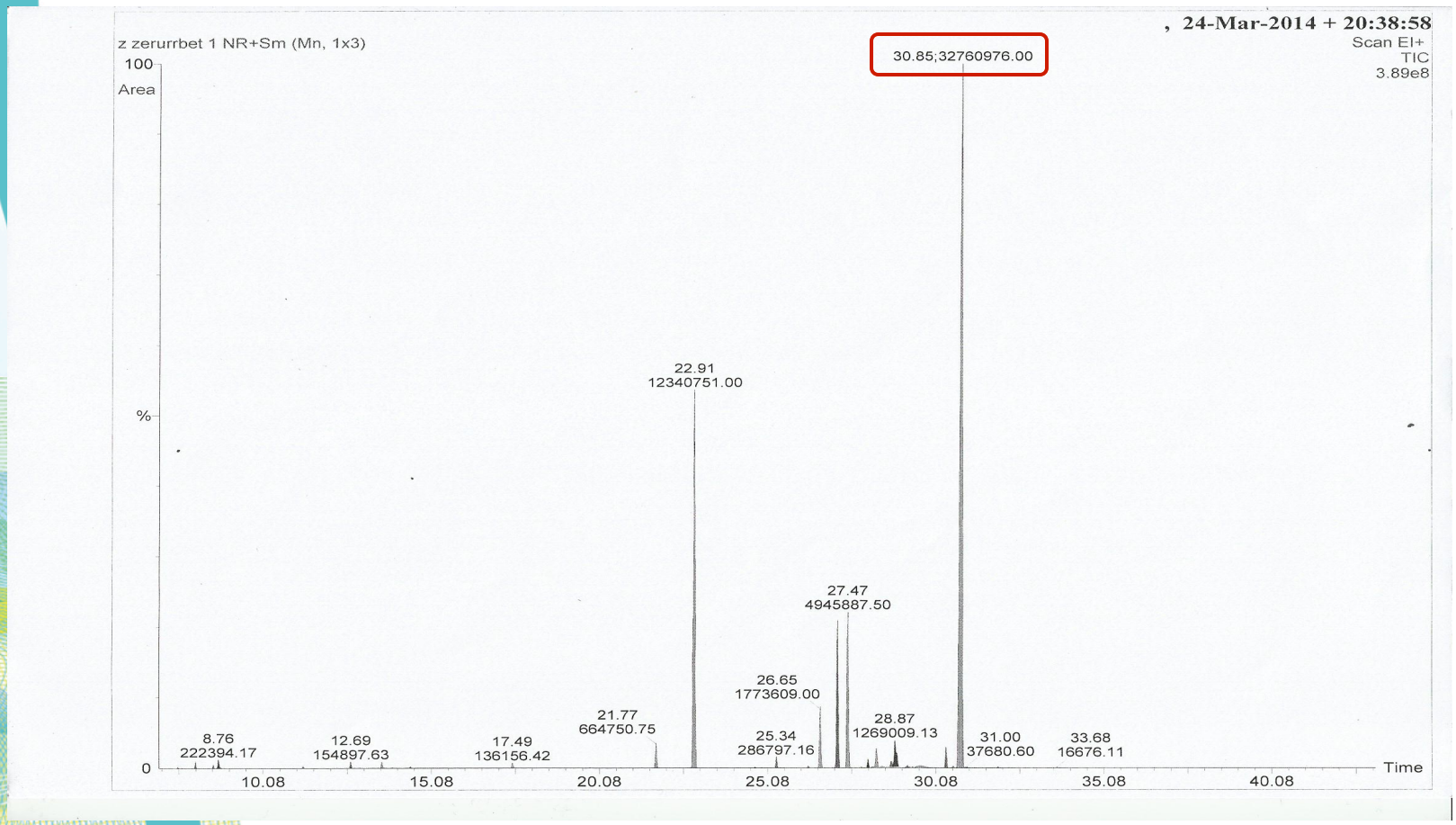
Mass spectrum of *Z. officinale* essential oil



Compounds Identified

Zingiber officinale	%
Eucalyptol	3.208
Cis-Verbenol	0.7319
β-Linalool	3.210
Borneol	10.057
Neral	32.52
Geranial	**37.82**
Limonene oxide	1.316
geranyl acetate	1.390
α-curcumene	4.327
β -sesquiphellandrene	2.246
β -farnesene/ α-bisabolol	0.654
β –eudesmol	0.8757
Trans nerolidol	1.637

Mass spectrum of Z. *zerumbet* essential oil



Compounds Identified

Zingiber zerumbet	%
Limonene	0.4072
Camphor	0.2836
Borneol/ Borneol acetate	0.2493
B-caryophyllene	1.217
α-Caryophyllene	22.598
β –Elemene	0.5252
Caryophyllene oxide	12.305
β-elemenone	2.324
2,6,10-Cycloundecatrien-1-one, 2,6,9,9-tetramethyl-/Zerumbone	**60.0**
α-farnesene	0.069
-1,3-Bis-(2-cyclopropyl, 2-methylcyclopropyl)-but-2-en-1-one	0.0305

Minimum Inhibitory Concentration (MIC) of Antibiotics

Microorganisms	Minimum Inhibitory Concentration (mg/mL)				
Gram-positive	Amp	Eryth	Pen	Strep	Tetra
• *S. aureus*	12.50	6.25	12.50	12.50	6.25
• *B. cereus*	12.50	6.25	6.25	3.13	6.25
Gram-negative					
• *E. coli*	12.50	25.00	12.50	12.50	50.00
• *P. aeruginosa*	12.50	12.50	25.00	50.00	25.00

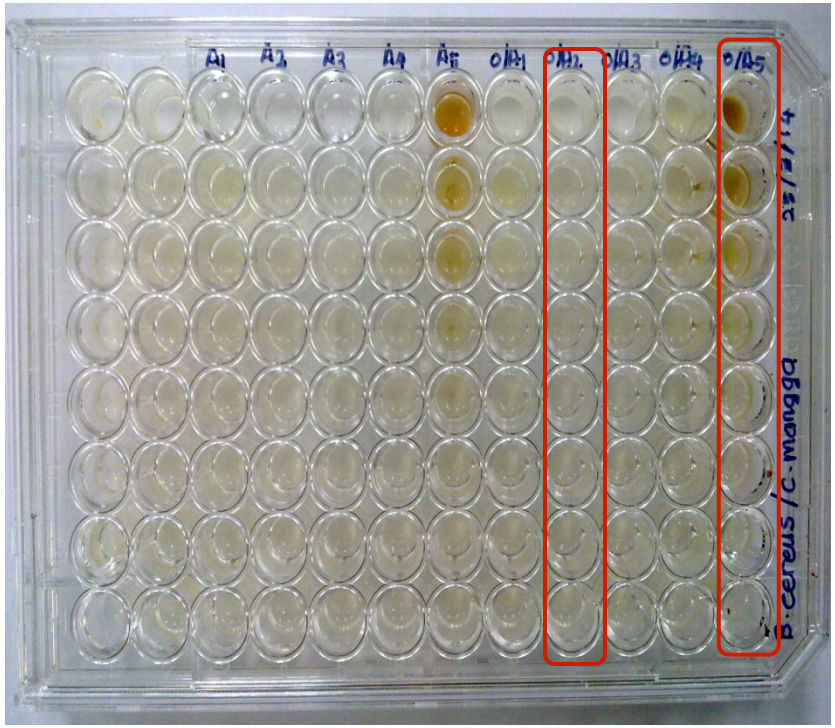
Minimum Inhibitory Concentration (MIC) of Essential Oils

Microorganisms	*C. mangga* (µL/mL)	*Z. officinale* (µL/mL)	*Z. zerumbet* (µL/mL)
Gram-positive			
• *S. aureus*	2.50	5.00	1.25
• *B. cereus*	5.00	1.25	5.00
Gram-negative			
• *E. coli*	1.25	5.00	2.50
• *P. aeruginosa*	5.00	5.00	5.00

* No bacterial inhibiton by DMSO

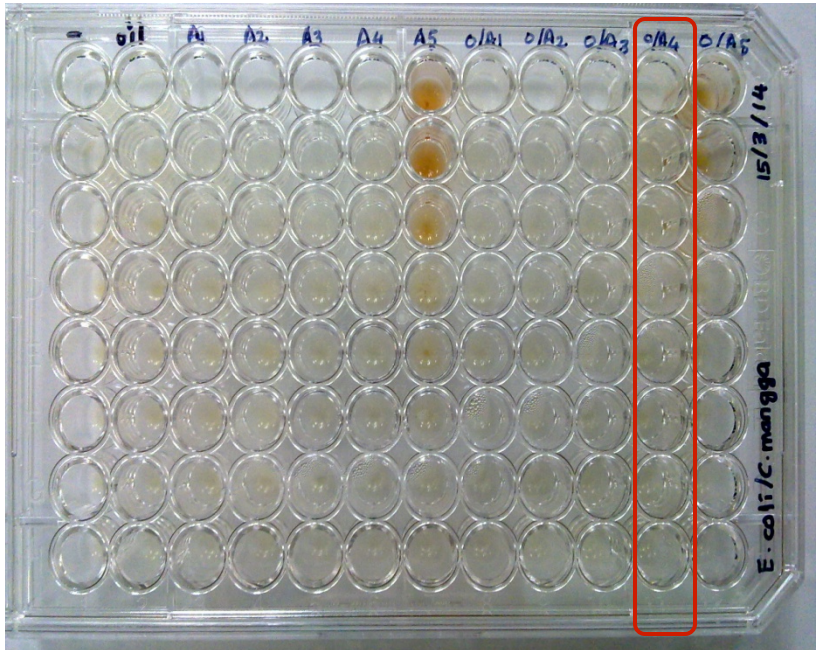
Curcuma mangga

	FIC index	Interaction
S. aureus		
Ampicillin/oil	7.20	Antagonism
Erythromycin/oil	1.00	Additive
Penicillin G/oil	3.60	Antagonism
Streptomycin/oil	3.60	Antagonism
Tetracycline/oil	4.20	Antagonism
B. cereus		
Ampicillin/oil	1.05	Indifferent
Erythromycin/oil	0.16	Synergy
Penicillin G/oil	1.35	Indifferent
Streptomycin/oil	7.80	Antagonism
Tetracycline/oil	0.17	Synergy



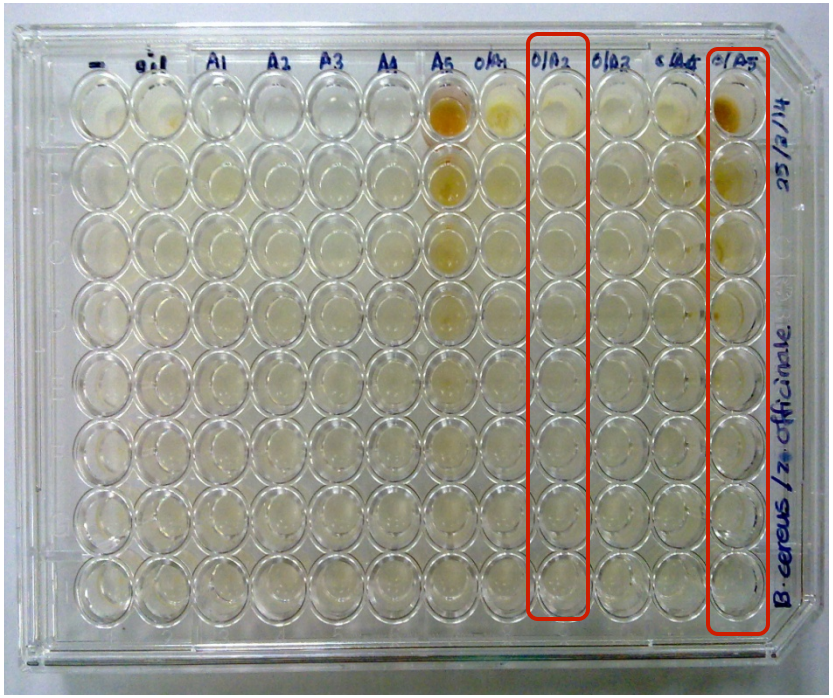
Curcuma mangga

	FIC index	Interaction
E. coli		
Ampicillin/oil	1.65	Antagonism
Erythromycin/oil	1.58	Indifferent
Penicillin G/oil	0.82	Additive
Streptomycin/oil	0.41	Synergy
Tetracycline/oil	12.30	Antagonism
P. aeruginosa		
Ampicillin/oil	8.40	Antagonism
Erythromycin/oil	8.40	Antagonism
Penicillin G/oil	3.60	Antagonism
Streptomycin/oil	13.20	Antagonism
Tetracycline/oil	7.20	Antagonism



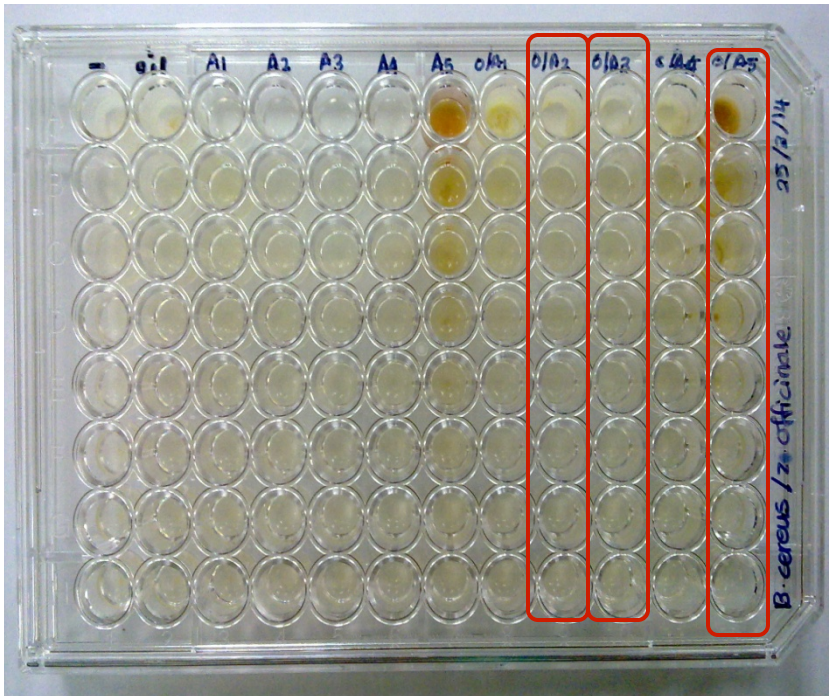
Zingiber officinale

	FIC index	Interaction
S. aureus		
Ampicillin/oil	4.20	Antagonism
Erythromycin/oil	0.68	Additive
Penicillin G/oil	1.00	Additive
Streptomycin/oil	8.40	Antagonism
Tetracycline/oil	5.40	Antagonism
B. cereus		
Ampicillin/oil	13.2	Antagonism
Erythromycin/oil	0.45	Synergy
Penicillin G/oil	7.20	Antagonism
Streptomycin/oil	4.20	Antagonism
Tetracycline/oil	0.45	Synergy



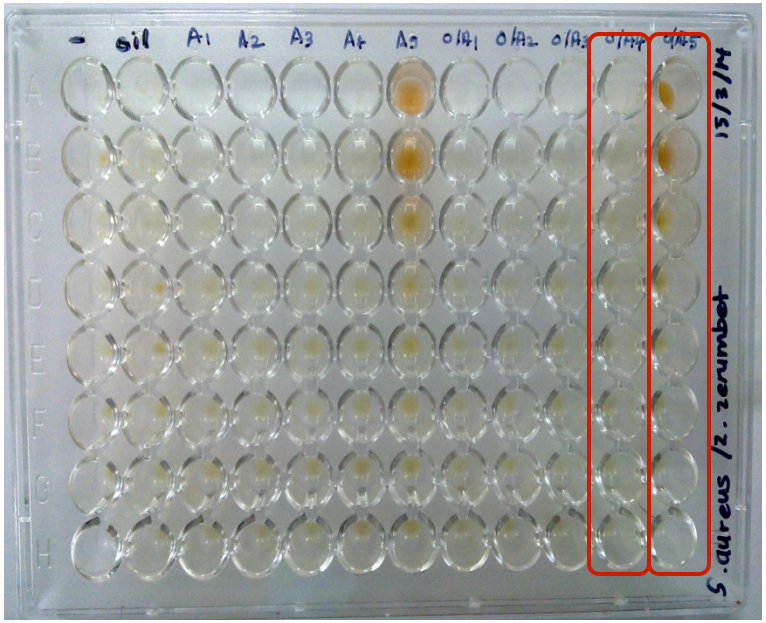
Zingiber officinale

	FIC index	Interaction
E. coli		
Ampicillin/oil	4.20	Antagonism
Erythromycin/oil	0.45	Synergy
Penicillin G/oil	0.13	Synergy
Streptomycin/oil	2.10	Antagonism
Tetracycline/oil	0.10	Synergy
P. aeruginosa		
Ampicillin/oil	2.10	Antagonism
Erythromycin/oil	2.10	Antagonism
Penicillin G/oil	1.80	Indifferent
Streptomycin/oil	13.20	Antagonism
Tetracycline/oil	3.60	Antagonism



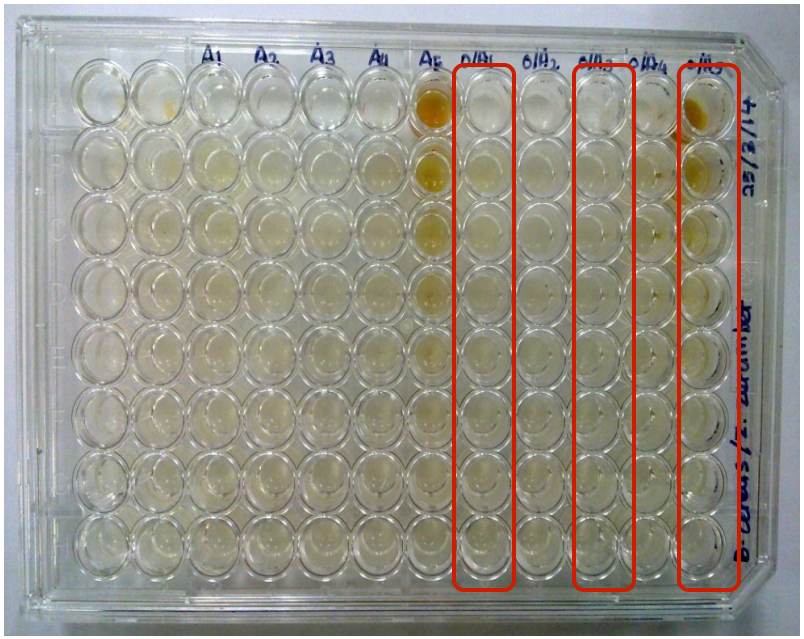
Zingiber zerumbet

	FIC index	Interaction
S. aureus		
Ampicillin/oil	6.60	Antagonism
Erythromycin/oil	0.90	Additive
Penicillin G/oil	13.20	Antagonism
Streptomycin/oil	0.41	Synergy
Tetracycline/oil	0.45	Synergy



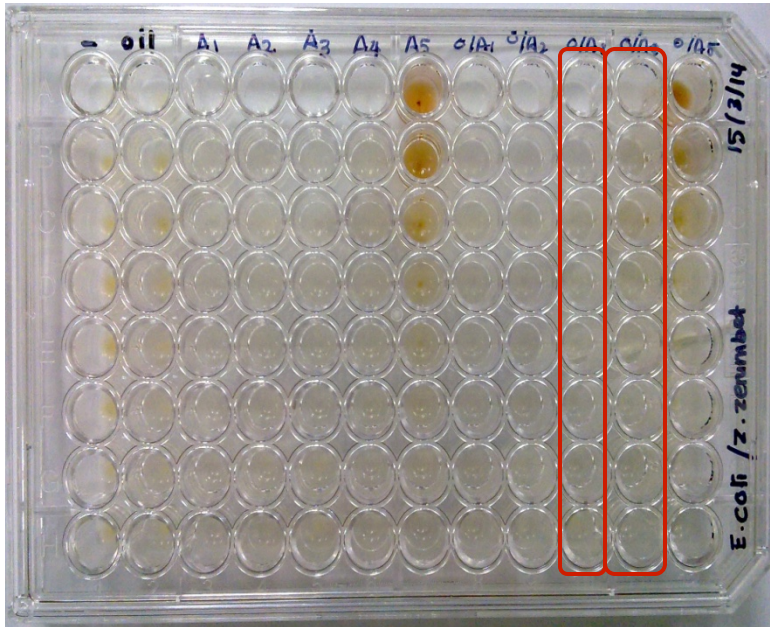
Zingiber zerumbet

	FIC index	Interaction
B. cereus		
Ampicillin/oil	0.13	Synergy
Erythromycin/oil	10.80	Antagonism
Penicillin G/oil	0.17	Synergy
Streptomycin/oil	1.95	Indifferent
Tetracycline/oil	0.17	Synergy



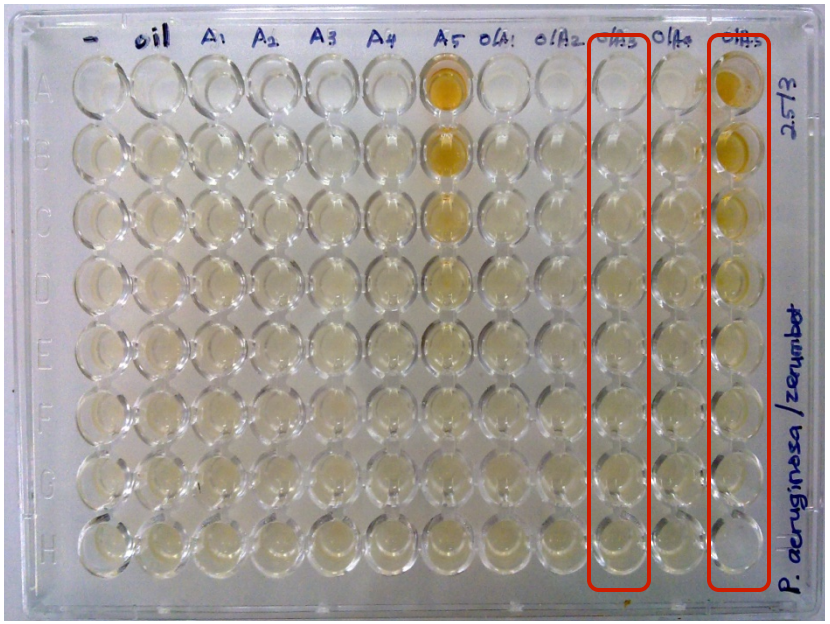
Zingiber zerumbet

	FIC index	Interaction
E. coli		
Ampicillin/oil	7.20	Antagonism
Erythromycin/oil	13.20	Antagonism
Penicillin G/oil	0.45	Synergy
Streptomycin/oil	0.45	Synergy
Tetracycline/oil	7.20	Antagonism



Zingiber zerumbet

	FIC index	Interaction
P. aeruginosa		
Ampicillin/oil	2.10	Antagonism
Erythromycin/oil	1.10	Indifferent
Penicillin G/oil	0.11	Synergy
Streptomycin/oil	2.64	Antagonism
Tetracycline/oil	0.11	Synergy



DISCUSSION

The higher resistance among Gram-negative bacteria could be due to differences in cell membrane of these bacterial groups (Okoh, 2010).

Gram-negative bacteria have an outer membrane surrounding the cell wall which restricts the diffusion of hydrophobic compounds (Kamazeri *et al*., 2012)

This study coincides with (Kamazeri *et al*., 2012): *C. mangga* contains caryophyllene oxide which contribute to its antibacterial properties.

Z. officinale possess moderate antibacterial activity as it contains caryophyllene oxide, geraniol, linalool which are known to possess antibacterial activity (Sivasothy *et al*., 2012)

Zerumbone from *Z. zerumbet* shows good antbacterial activity against Gram-positive bacteria
(Malek, 2012)

The mechanisms by which essential oils can inhibit microorganisms has not been fully understood but in part may be due to their hydrophobicity
(Helander *et al*., 1998)

Essential oils accumulate in lipid bilayer alters membrane integrity, thus membrane becomes more permeable to antibiotics
(Nicolson *et al*., 1999)

The membrane permeability leads to loss of vital cell contents which eventually leads to cell death (Burt, 2004)

CONCLUSION

- All three essential oils exhibit antibacterial properties.
- *Z. zerumbet* has the most potent antibacterial property
- Gram-positive bacteria are more susceptible to essential oils compared to Gram-negative bacteria.
- Essential oils are able to synergize with antibiotics against bacteria.

FUTURE RECOMMENDATIONS

- Essential oils may be used as adjuvant in antibiotic therapy.
- Combination of essential oils might be possible to see if they could produce a stronger synergistic effect.
- *In vivo* studies could be furthered to see if there are any toxicity in the combination.

ACKNOWLEDGEMENT

- **Supervisor**: Assoc. Prof. Dr Deny Susanti
- **Lecturers**: Dr. Norazian Mohd Hassan
- **Beloved parents**
- **FYP Cooridnator**: Dr Mardhiah Mohamad
- **Lab Assistants**: Br Muzammil, Sr. Mueizzah
- **Dearest family and friends**
- This Final Year Project has been presented during the 5th Biomedical Symposium, UKM (10th May 2014).

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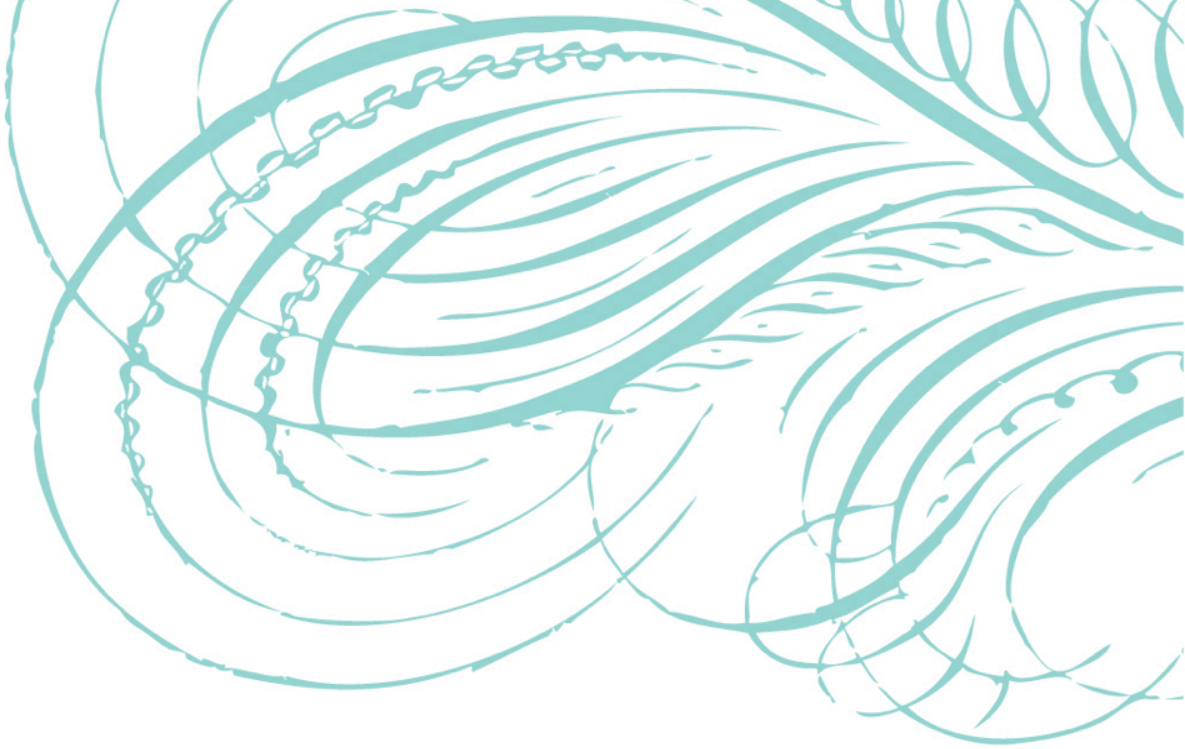

Thank You