

In-Vitro Antibacterial Activity of Phytochemicals Against *Escherichia coli* and *Mycoplasma gallisepticum*



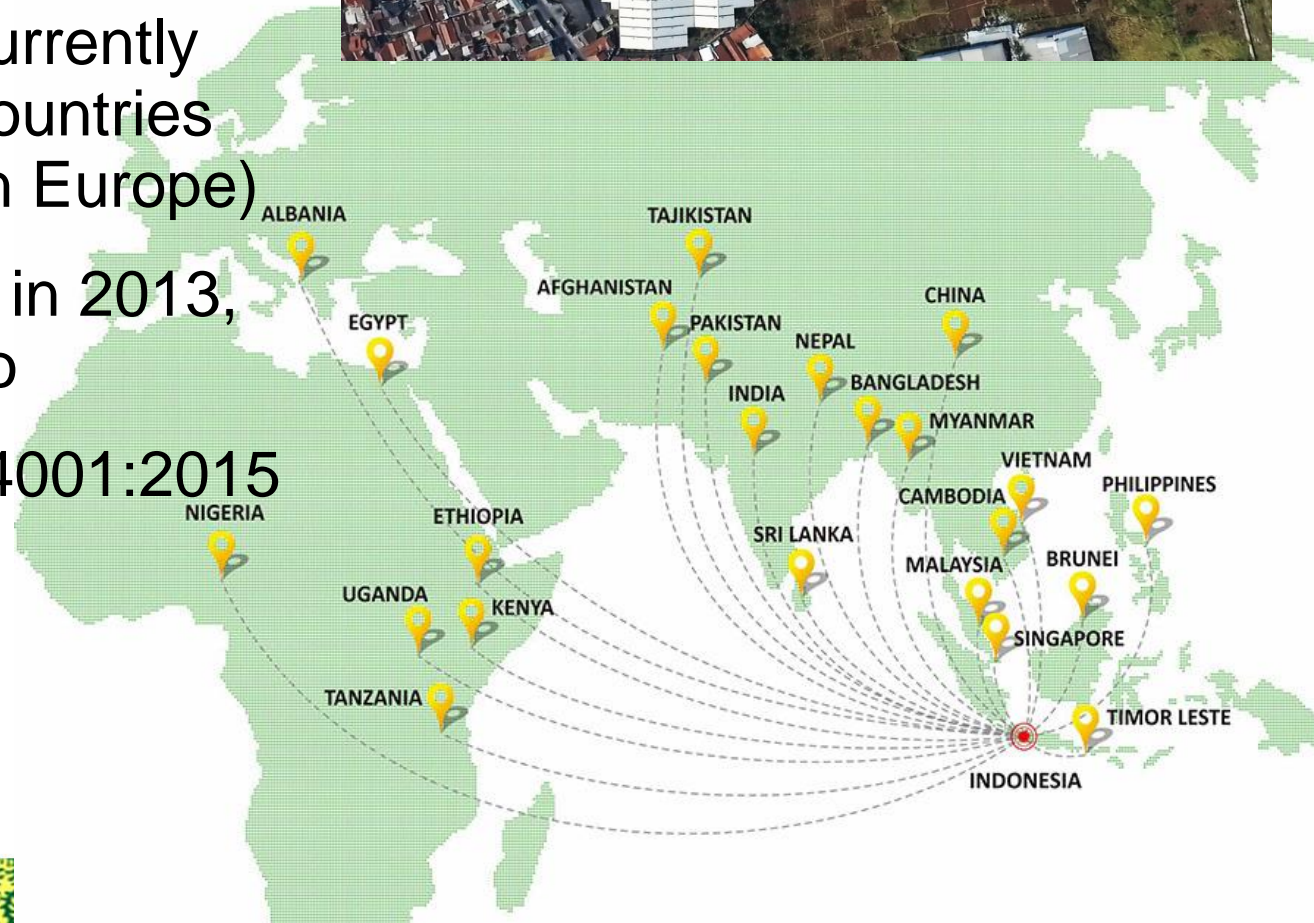
ATA Bangkok 2019

Elvina J. Jahja
Animal Health
Research & Development

Who We Are

Medion Farma Jaya,
Bandung - Indonesia

- Established in 1976
- First export in 1998, currently have exported to 23 countries (Asia, Africa & Eastern Europe)
- Launched **mediherba** in 2013, 10 products in portfolio
- ISO 9001:2015 and 14001:2015 certified



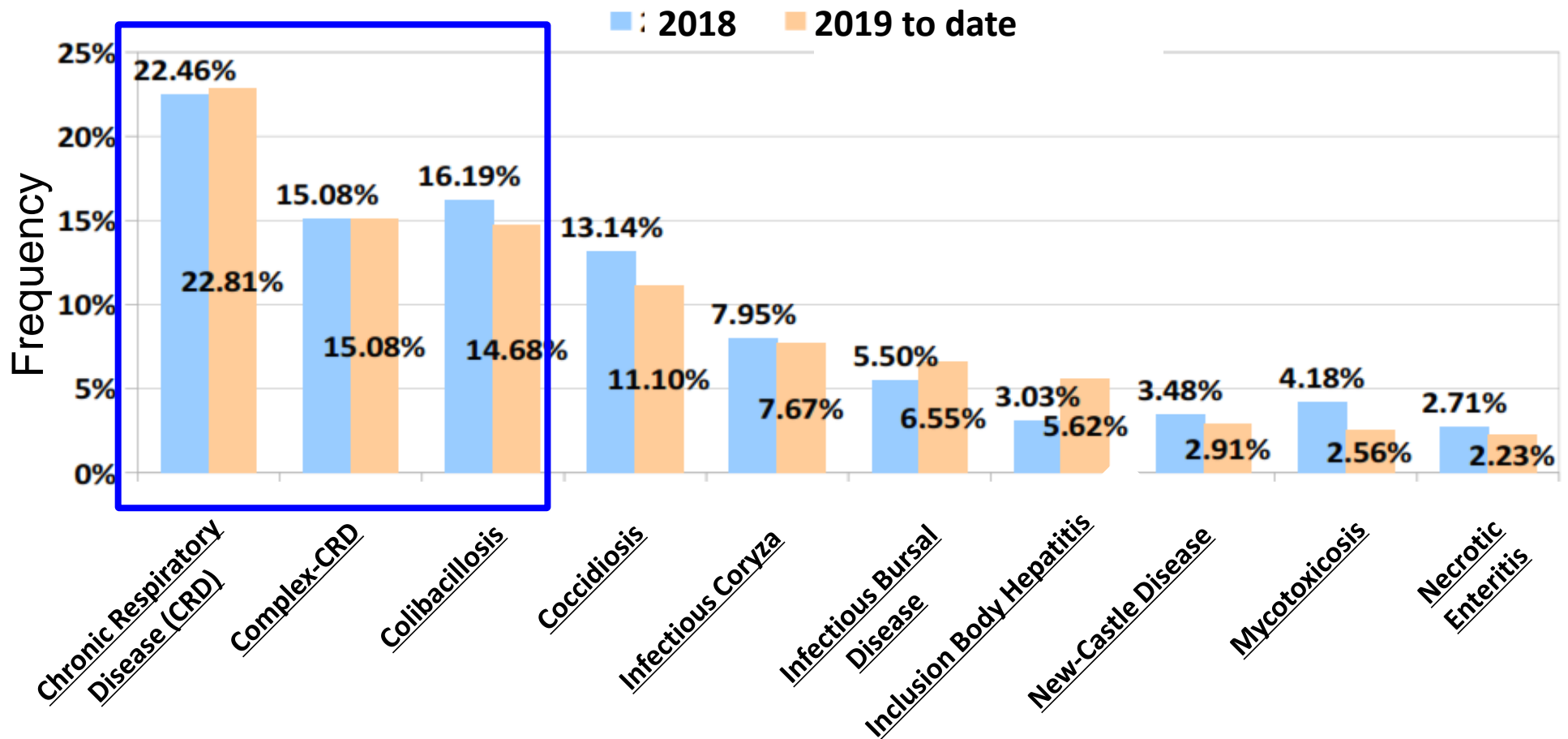


Who our partner is

Department of Pharmacology and Clinical Pharmacy,
Bandung Institute of Technology (ITB), Indonesia

- Part of School of Pharmacy in ITB
- Establish since October 6th, 1947
- Consists of three subgroups : Pharmacology – Toxicology, Immunology, and Clinical Pharmacy
- Focus in: Education, Research, and Pharmaceutical Care

Common Poultry Disease in Indonesia



(Technical Education and Consultation of Medion, 2019)

Plant Extracts as an Alternative



Indonesia has
 \pm 30,000 plants
diversity



\pm 7.500 among
them are
medicinal plants



Only 300 plants that routinely used in traditional
medicine industry
(Indonesian NADFC, 2016)

Objectives

To determine **in-vitro antibacterial activity** of 10 Indonesian plant ethanol-extracts and their combination against ***Escherichia coli*** and ***Mycoplasma gallisepticum***.

Plant Materials



Sappan wood



Guava leaves



Red ginger



Elephant ginger



Nutmeg fruit



Roselle calyx



Indonesian bay leaf



Corn silk



Lesser galangal



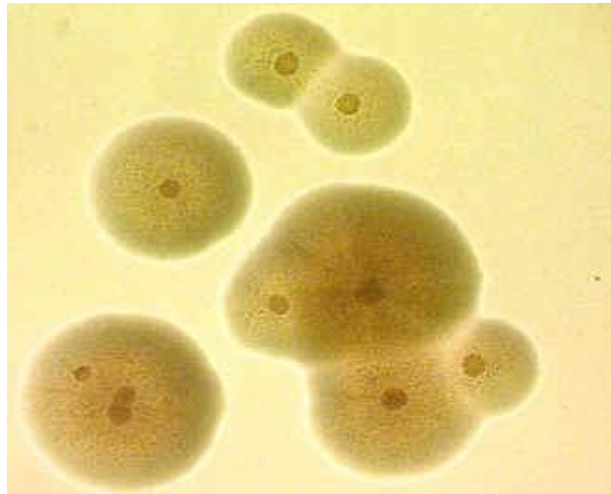
Galangal rhizome

Bacteria

- *Escherichia coli* serotype O78.K80.H12 (ATCC 43896)
- *Mycoplasma gallisepticum* strain S6 (ATCC 15302)



Escherichia coli



Mycoplasma gallisepticum

Methods

Preparation of Plant Extracts

- Reflux extraction using 96% ethanol at 65° C for 2 hours



1. Antibacterial Activity Screening against *E. coli*

Disk diffusion method



2. Minimum Inhibitory Concentration against *E. coli*

Single extract: broth microdilution method

3. Combination effect of extracts: paper ribbon method



4. Minimum Inhibitory Concentration against *M. gallisepticum*

Single & combination extracts: broth color changing method

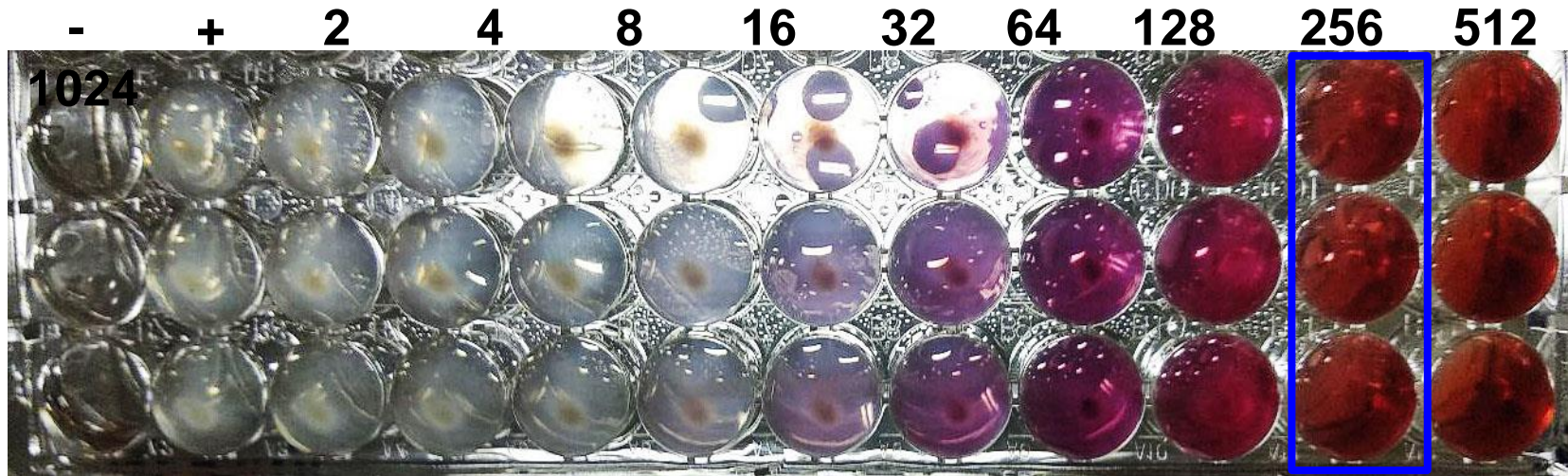
(Thilagavathi et al, 2017)

Results

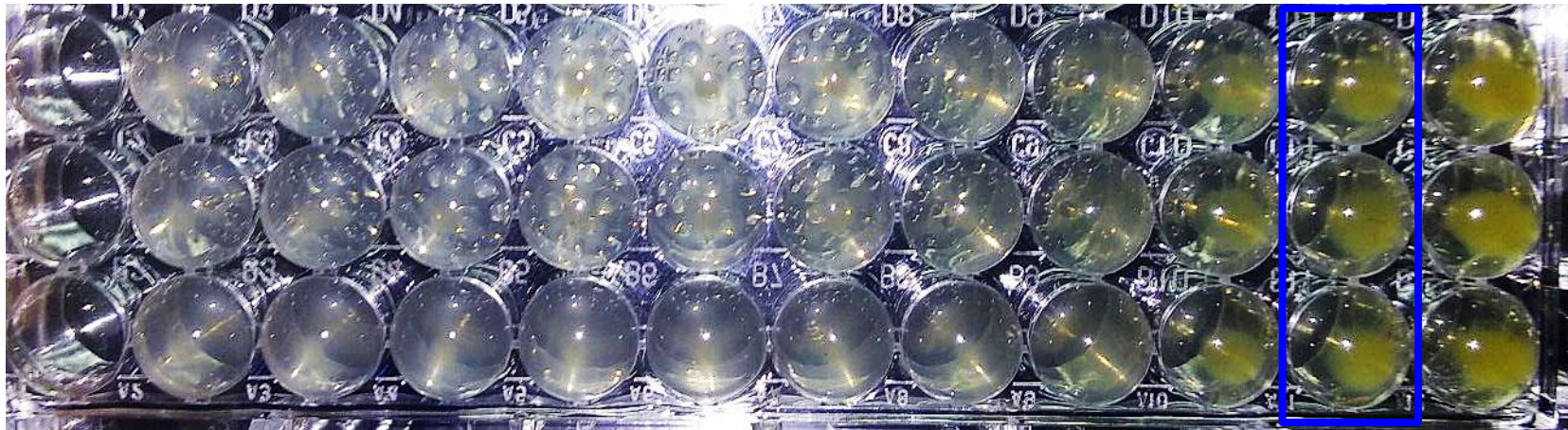
Antimicrobial screening & MIC against *E. coli*

Plant Extracts	Activity (1-5%)	MIC ($\mu\text{g/ml}$)
Sappan wood	+	256
Guava leaves	+	256
Red ginger rhizome	+	512
Elephant ginger	+	1024
Nutmeg fruit	+	1024
Roselle calyx	+	5120
Indonesian bay leaf	+	10240
Corn silk	+	>10240
Lesser galangal rhizome	+	>10240
Galangal rhizome	+	>10240

MIC against *E. coli*

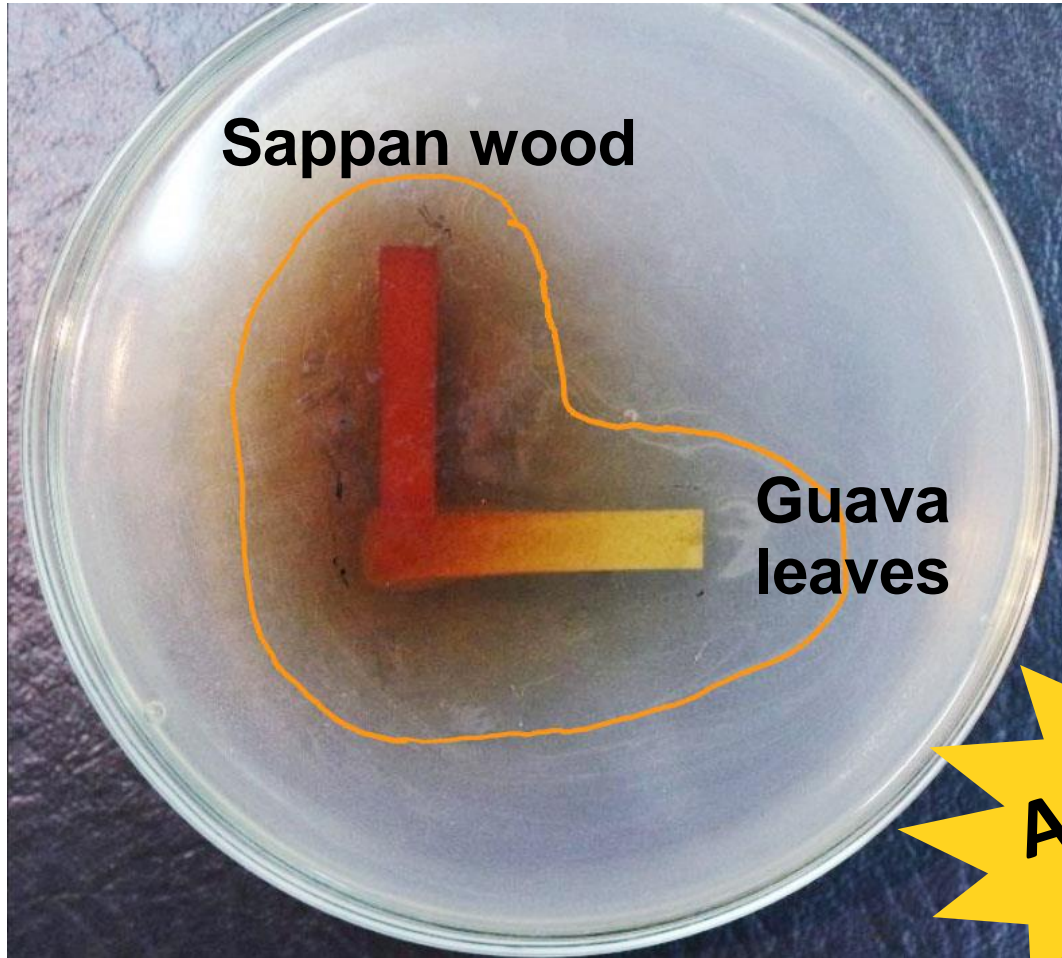


Sappan wood extract (µg/ml)



Guava leaves extract (µg/ml)

Combination Extracts against *E. coli*



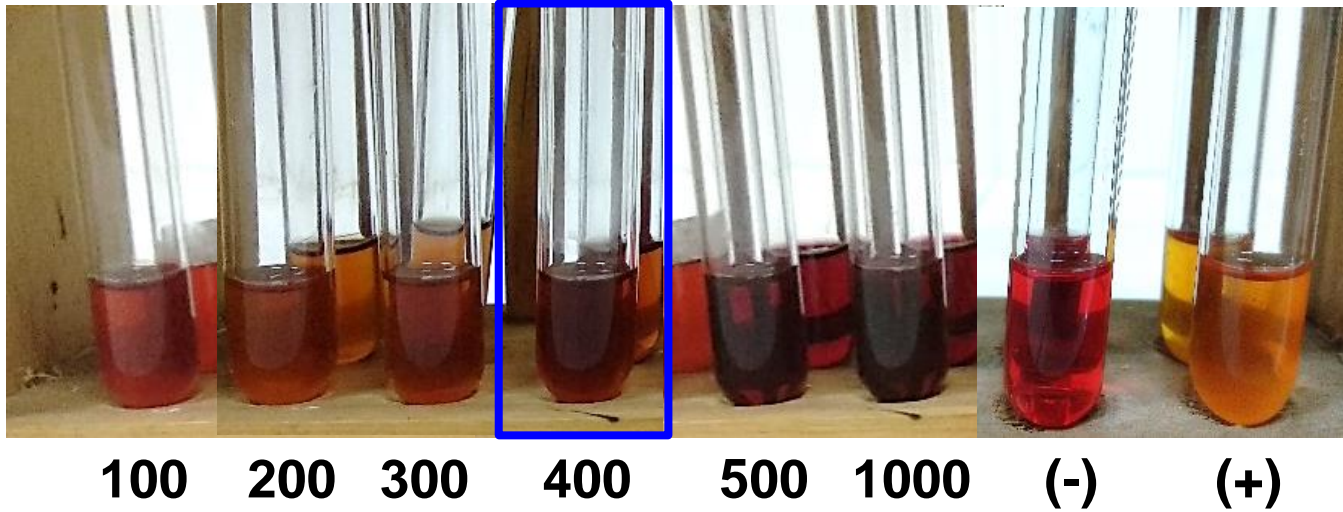
Additive

Extract concentration at 10%

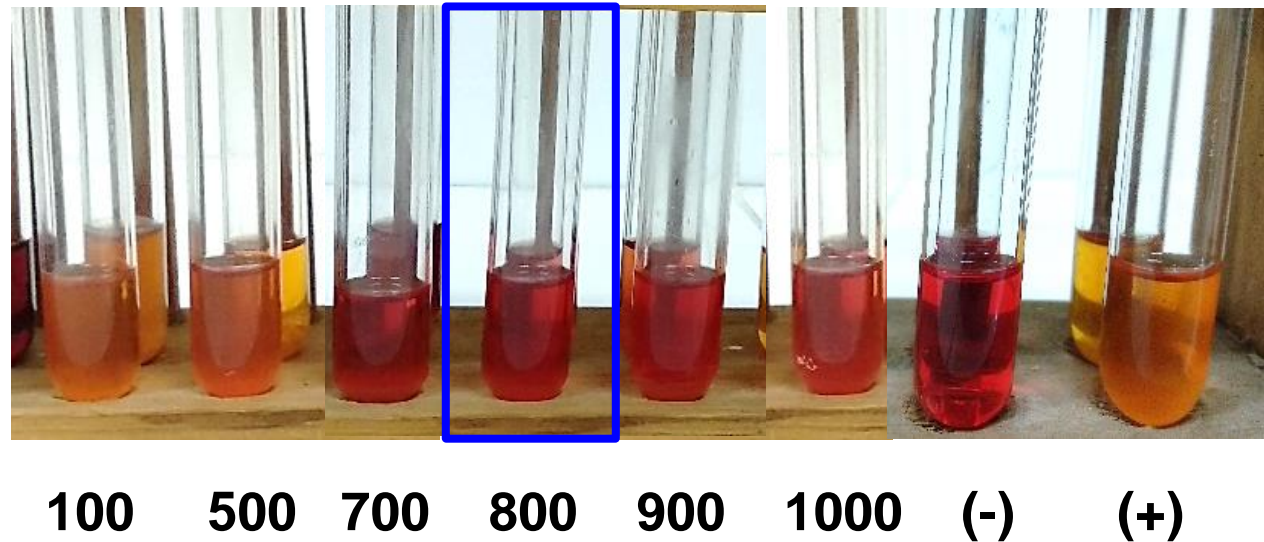
Minimum Inhibitory Concentration against *M. gallisepticum*

Extracts	MIC ($\mu\text{g/ml}$)
Sappan wood	400
Red ginger rhizome	800
Guava leaves	>1000
Nutmeg fruit	>1000
Roselle calyx	>1000

MIC against *M. gallisepticum*

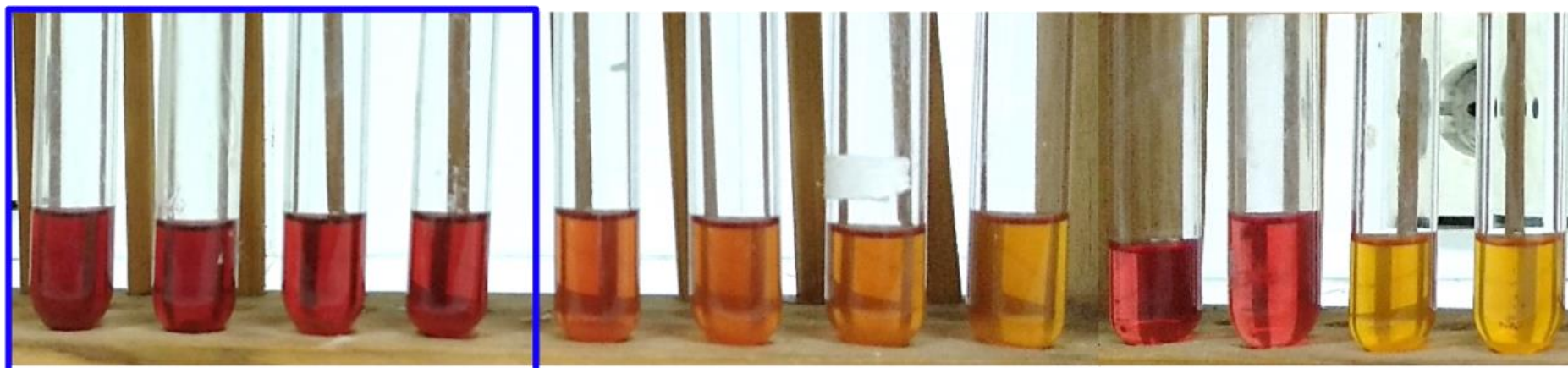


Red ginger rhizome ($\mu\text{g/mL}$)



Combination Extracts against *M. gallisepticum*

Combo #	Concentration of Extracts ($\mu\text{g/ml}$)		Effects
	Sappan wood	Red ginger	
1	400	800	Additive
2	200	400	Additive
3	100	200	No effect
4	50	100	No effect



Combo #1

Combo #2

Combo #3

Combo #4

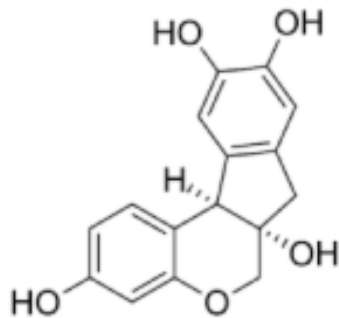
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Active Substance that has Antibacterial Activity



Sappan wood

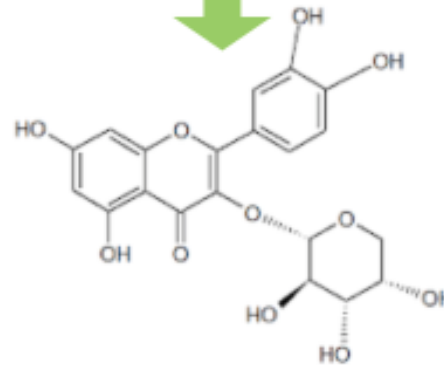


Brazilin

(Nirmal and Panichayupakaranant, 2015)



Guava leaves

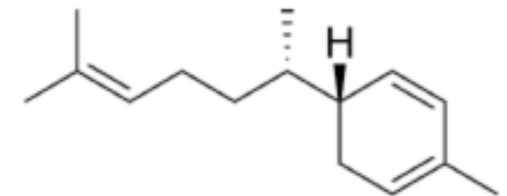


Guajaverin

(Goncalves, 2008)



Red ginger



Zingiberene

(Malu, et al, 2008)

Antimicrobial Mechanism of Action

Plant Extract	Mechanism of Action	Reference
<i>Caesalpinia sappan</i>	Inhibiting DNA and protein synthesis	Nirmal et al, 2015
<i>Psidium guajava</i>	Penetrate the lipid bilayer of the cell membrane, rendering it more permeable, leading to the leakage of vital cell contents	Biswas et al, 2013
<i>Zingiber officinale</i> <i>Var Rubrum</i>	Affecting bacteria cell wall permeability and release of intracellular constituents	Hasan HA et al, 2012

Summary

- Ethanol extracts of **sappan wood** and **guava leaves** showed additive antibacterial activity against *Escherichia coli*
- **Sappan wood** and **red ginger rhizome** showed additive antibacterial activity against *Mycoplasma gallisepticum*
- Highly potential phytochemicals to be used in commercial poultry farms
- Need to determine most effective combination and cost-efficient dose in poultry (in vivo)

Acknowledgement



Prof. dr. Elin Yulinah S.



Dhyhan Kusuma A., M.Si., Apt.



Riana Yuliana, S. Si., Apt.



Welinda T.S., S. Farm., Apt.



Thank You

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