# How Effective Are Natural Antimicrobials At Inhibiting The Growth Of Bacteria?

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# **BACKGROUND** •





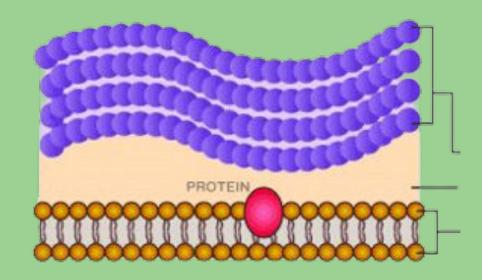
# **BACKGROUND INFO**

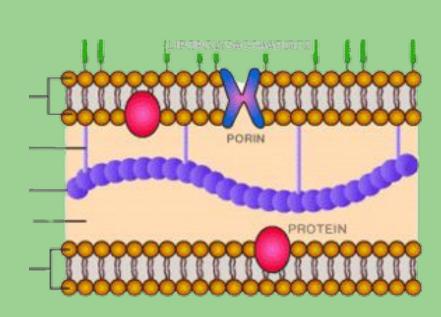
#### **Our Aims:**

- Investigate how effective different natural antimicrobial agents are at inhibiting the growth of bacteria
- Come to conclusive results on the best methods to keep surfaces and foodstuffs sanitary and safe
- Improve our understanding on the antimicrobial properties of natural compounds

#### **Opportunities:**

- Addressing resistance of bacteria to pharmaceutical antibiotics
- Finding sustainable sources of antibiotics
- Exploring alternative treatments for bacterial infections that can decrease side-effects and complications





# WHY LACTOBACILLUS?

**Gram Positive** (Lactobacillus acidophilus)

**Gram Negative** (Escheria coli)

# GOAL: SUSTAINABILITY

## Addressing:

Toxic chemicals

### Wasteful production





Impacts on health





**Cost-effectiveness** 



# **ANTIMICROBIALS**

#### Garlic

Allicin - interferes with enzymes

#### Clove

Eugenol - affects membrane permeability and cell growth

#### **Orange Peel**

Lime Peel

Flavonoids - denature proteins and inhibit cell growth

> Saponin - affects membrane permeability

White Vinegar

Acetic acid unfavourable pH

Honey

High sugar contentdehydration of cells

#### Oregano

Thymol and carvacrol disrupts cell membrane and wall

#### Lemongrass

Citral - changes ATP concentration, cell membrane and pH



# METHOD





# OUR METHOD



#### **Extract Making**

Dry ingredients cut or crushed with pestle and mortar

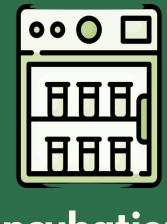
5g of antimicrobial + 15g of boiled water



#### Streaking

*Lactobacillus acidophilus* streaked onto agar gel

Filter paper soaked in antimicrobial in the centre



Incubation

Petri dishes placed in incubator set at 37°C for 24 hours





Area of bacterial growth measured and recorded





# **PATHOGENIC BACTERIA CULTURES**

**Problem:** Slim chance of this bacterium causing illness and spareding

#### Solutions:

• Treat all microorganisms as if they are pathogens





- Severely immunocompromised/ill students should 0 consult with a medical professional
- Any samples or solutions used that may be 0 hazardous was labelled with a clearly visible and sufficiently detailed warning
- All waste in contact with bacteria should be discarded into a biohazard bag

# **GENERAL LAB** SAFETY

**Problem:** Broken glassware and equipment can cause injury

#### Solutions:

- Place all equipment on flat, level surfaces
- 0
  - into a properly labelled waste bag

#### **Problem:** Hazardous chemicals Solution:

- bacteria and any spills.
- 0 contact with chemicals and bacteria.

Ensure that all broken glassware is discarded

• Lab coats should be worn to protect skin and clothing from being completely exposed to

Disposable latex gloves should be worn to limit

# S Z S 0

## petri dish -

## bacterial growth -

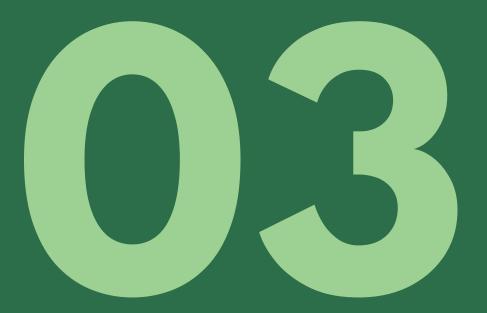


# THE SETUP

# filter paper with antimicrobial

agar gel

# CONCLUSION



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Natural antibiotic	Width (mm)				Length (mm)				Average diameter (mm)				Area (mm <sup>2</sup> )			
	<b>T1</b>	<b>T2</b>	Т3	Ā	<b>T1</b>	<b>T2</b>	Т3	Ā	<b>T1</b>	<b>T2</b>	<b>T3</b>	Ā	<b>T1</b>	<b>T2</b>	Т3	Ā
Garlic	18	9	9	12	10	7	8	8	-	8	9	9	180	50	64	98
White vinegar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Honey	7	8	12	9	7	7	14	9	7	8	13	9	39	50	133	74
Clove	6	7	7	7	5	7	7	6	6	7	7	7	28	38	38	35
Oregano	11	40	12	21	11	24	12	16	11	-	12	8	95	960	113	389
Orange peel	19	0	13	11	17	0	12	10	18	0	13	10	254	0	133	129
Lime peel	55	23	47	42	22	18	27	22	-	-	-	-	1210	414	1269	964
Lemongrass	18	21	21	20	20	23	20	21	19	22	21	31	284	380	346	337
CONTROL	16				17				17				227			

# **Conclusion:**

White vinegar inhibits the growth of Lactobacillus acidophilus most effectively.

## Implications

- Can prevent growth of other bacteria (E. coli, Salmonella etc.)
- Gram negative bacteria can be more susceptible (thinner walls)
- Bacteria are inhibited best by low pH
- Can interfere with good bacteria/probiotics

## CONCLUSION

# **Sources of Error:**

- Shape of the bacteria colonies:
  - Inorganic shapes
  - Difficulty measuring accurate area
- Poor sealing of petri dishes:
  - Contamination of other microorganisms
  - Competition for resources affecting bacteria's growth



## **EVALUATION**









#### **References**:

[1] Bintsis, T. (2017) Foodborne pathogens, AIMS microbiology. U.S. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6604998/ (Accessed: 3 October 2023).

[2] Ibrahim, S., Wei Hoong, L., Lai Siong, Y., Mustapha, Z., C. W. Zalati, C. W. S., Aklilu, E., Mohamad, M. and Kamaruzzaman, N. F. (2021) Prevalence of Antimicrobial Resistance (AMR) Salmonella spp. and Escherichia coli Isolated from Broilers in the East Coast of Peninsular Malaysia, MDPI. Multidisciplinary Digital Publishing Institute. Available at: <a href="https://www.mdpi.com/2079-6382/10/5/579">https://www.mdpi.com/2079-6382/10/5/579</a> (Accessed: 4 October 2023).

[3] Bhat, S. A., Sher, F., Kumar, R., Karahmet, E., Haq, S. A. U., Zafar, A. and Lima, E. C. (2022) Environmental and health impacts of spraying COVID-19 disinfectants with associated challenges, Environmental science and pollution research international. U.S. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8486161/ (Accessed: 4 October 2023).

[4] Oji, I. (2022) How Cleaning Products Affect The Environment, Carlson Building Maintenance. <u>https://carlsonbuilding.com/wp-content/uploads/2020/12/CBM-New-Logo-m.png.</u> Available at: <u>https://carlsonbuilding.com/how-cleaning-products-affect-the-environment (Accessed: 8 October</u> 2023).

[5] Essential Malaysian ingredients (2010) The Guardian. Guardian News and Media. Available at: https://www.theguardian.com/lifeandstyle/2010/dec/28/essential-malaysian-ingredients (Accessed: 5 October 2023).

[6] Old Wives' Tales and Garlic as an Antibiotic: Are Microbial Myths Fact or Fiction? (no date) ASM.org. Available at: https://asm.org/Articles/2019/July/Old-Wives-Tales-and-Garlic-as-an-Antibiotic-Are-Mi (Accessed: 5 October 2023).

[7] ABC News (2018) Does vinegar really kill household germs?, Does vinegar really kill household germs? - ABC News. ABC News.

[8] Albaridi, N. A. (2019) Antibacterial Potency of Honey, International journal of microbiology. U.S. National Library of Medicine. Available at: <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6589292">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6589292</a> (Accessed: 5 October 2023).

[9] Nuñez, L. and Aquino, M. D. (2012) Microbicide activity of clove essential oil (Eugenia caryophyllata), Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]. U.S. National Library of Medicine. Available at: <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3769004">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3769004</a> (Accessed: 5 October 2023).

[10] Fournomiti, M., Kimbaris, A., Mantzourani, I., Plessas, S., Theodoridou, I., Papaemmanouil, V., Kapsiotis, I., Panopoulou, E. E. and Alexopoulos, A. (2015) Antimicrobial activity of essential oils of cultivated oregano (Origanum vulgare), sage (Salvia officinalis), and thyme (Thymus vulgaris) against clinical isolates of Escherichia coli, Klebsiella oxytoca, and Klebsiella oxytoca, and Klebsiella oxytoca, and Klebsiella pneumoniae, Microbial Library of Medicine. Available at: <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4400296">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4400296</a> (Accessed: 5 October 2023).

[11] Wang L., Zhao X., Zhu C., Xia X., Qin W., Li M., Wang T., Chen S., Xu Y., Hang B., Sun Y., Jiang J., Richard L.P., Lei L., Zhang G., Hu J (2017) Thymol kills bacteria, reduces biofilm formation, and protects mice against a fatal infection of Actinobacillus pleuropneumoniae strain L20, Veterinary microbiology. U.S. National Library of Medicine. Available at: <a href="https://pubmed.ncbi.nlm.nih.gov/28619145">https://pubmed.ncbi.nlm.nih.gov/28619145</a> (Accessed: 5 October 2023).

[12] Wijesundara, N. M., Lee, S. F., Cheng, Z., Davidson, R. and Rupasinghe, H. P. V. (2021) Carvacrol exhibits rapid bactericidal activity against Streptococcus pyogenes through cell membrane damage, Nature News. Nature Publishing Group. Available at: <a href="https://www.nature.com/articles/s41598-020-79713-0">https://www.nature.com/articles/s41598-020-79713-0</a> (Accessed: 5 October 2023).

[13] Herawati, D., Ekawati E.R., Yusmiati, S.N.H., Identification of Saponins and Flavonoids in Lime (Citrus aurantifolia) Peel Extract (2020). Available at: <a href="http://www.ieomsociety.org/detroit2020/papers/724.pdf">http://www.ieomsociety.org/detroit2020/papers/724.pdf</a> (Accessed: 5 October 2023).

[14] Schweitzer, B., Balázs, V. L., Molnár, S., Szögi-Tatár, B., Böszörményi, A., Palkovics, T., Horváth, G. and Schneider, G. (2022) Antibacterial Effect of Lemongrass (Cymbopogoncitratus) against the Aetiological Agents of Pitted Keratolyis, Molecules (Basel, Switzerland). U.S. National Library of Medicine. Available at: <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8878996">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8878996</a> (Accessed: 5 October 2023).

[15] Shi, C., Song, K., Zhang, X., Sun, Y., Sui, Y., Sui, Y., Chen, Y., Jia, Z., Sun, H., Sun, Z. and Xia, X. (2016) Antimicrobial Activity and Possible Mechanism of Action of Citral against Cronobacter sakazakii, PloS one. U.S. National Library of Medicine. Available at: <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4945043">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4945043</a> (Accessed: 6 October 2023).

#### Further references:

Quinto EJ, Caro I, Villalobos-Delgado LH, Mateo J, De-Mateo-Silleras B, Redondo-Del-Río MP. (2019) "Food Safety through Natural Antimicrobials" National Library of Medicine. Available at: <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6963522/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6963522/</a> (Accessed: 27 September 2023)



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