# Zoochemical Analyses and *In vitro* Antimicrobial Activity of Crude Methanolic extracts of *Perna viridis*


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# Project Description

*(Image credit: Review on Antimicrobial Resistance (2014))*


*(Image credit: The American Journal of Pathology/Ritter et al. (2013))*

**Problems:**

- Antimicrobial resistance
- Fungal infections
- Increasing demands of antibiotics

**"The invasive *Tahong*"**

- Reproduces fast despite of environmental stresses (e.g. typhoons)
- A major industry in coastal communities but unstable market

**Aims of the project:**

- to discover commercially useful biomolecules from mussels
- this study focused on the antimicrobial activity of the crude methanolic extracts as well as the zoochemicals present

# Objectives

1. To screen for the zoochemicals present in the crude methanolic extracts of *P. viridis* samples;

2A. to determine the antibacterial activity of *P. viridis* extracts against gram-negative bacteria namely *Escherichia coli* and *Pseudomonas aeruginosa* and gram-positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis;*

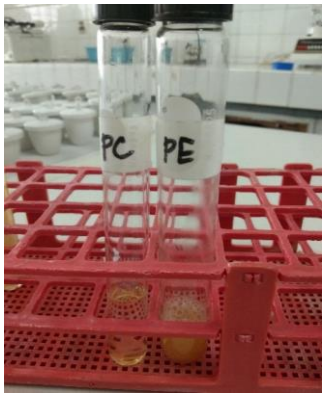
2B. to determine the antifungal activity of *P. viridis* extracts against *Rhizopus sp.*, *Lasiodiplodia theobromae* , and *Colletotrichum capsici;* and

3. to determine the minimum inhibitory concentration of *P. viridis* extracts using broth microdilution method.

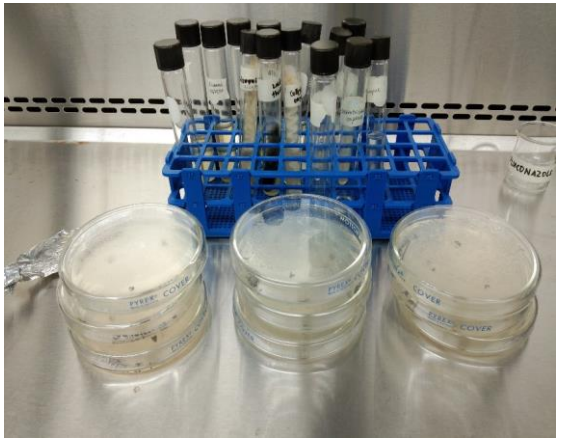
# Materials and Methods

## Extraction

## Zoochemical Analyses (Qualitative and TLC)




## Antibacterial, Antifungal and MIC evaluation



## Data Analysis

# Results

**Table 1.** Qualitative Zoochemical Screening of the crude methanolic extracts of the green mussel *P. viridis*

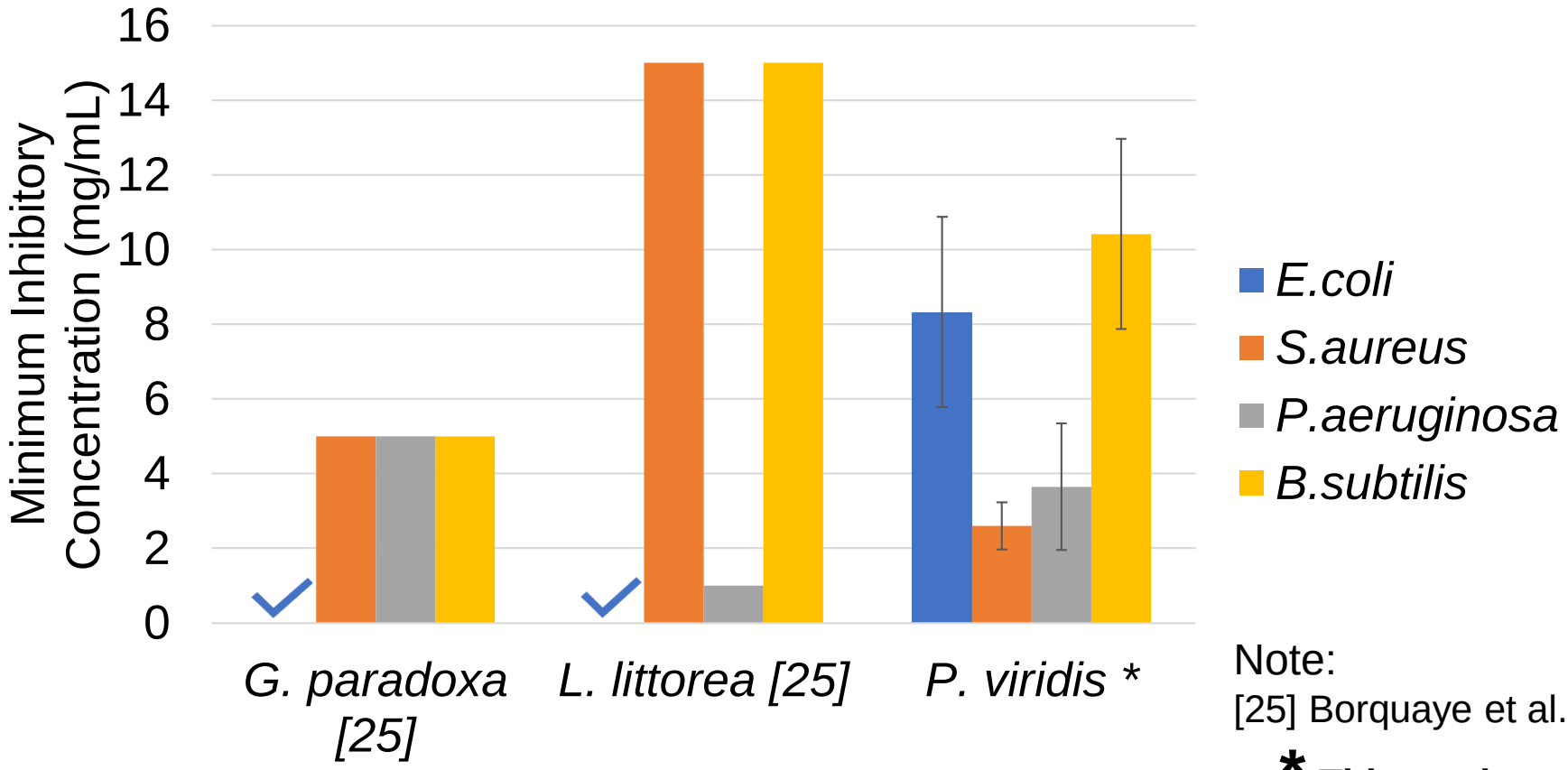
| Zoochemical | Observation | Result |
|---|---|---|
| **Saponins** | Two centimeters honeycomb-like froth | + |
| **Polyphenols** | Brown precipitate | + |
| **Flavonoids** | Pale Yellow Coloration | - |
| **Alkaloids** | Yellow Precipitate | + |
| **Sterols** | Green Coloration | + |
| **Terpenoids** | Yellow layers with reddish interface | + |
| **Cardiac glycosides** | Pale green coloration | - |

# Results

**Table 4**. Antimicrobial Activity of the crude methanolic extracts of *P. viridis*

| | Zone of Inhibition (mm) | |
|---|---|---|
| **Gram negative** | **MeOH extracts (100 mg/ml)** | **Streptomycin (10 mg/mL)** |
| *E.coli* | 18.00±1.25 | 23.67±0.47 |
| *P. aeruginosa* | 19.00±0.82 | 21.33±0.47 |
| **Gram positive** | | |
| *S.aureus* | 19.33±0.47 | 25.33±1.70 |
| *B.subtilis* | 17.67±0.94 | 21.67±0.94 |
| **Fungi** | **MeOH extracts (100 mg/ml)** | **Fluconazole (10 mg/mL)** |
| *Rhizopus sp.* | - | 8.0±0.82 |
| *C. capsici* | - | 13.0±1.41 |
| *L. theobromae* | 7.33±0.94 | 9.33±0.47 |

# Results




Note:
[25] Borquaye et al. 2016
* **This study**
✓ tested

**Figure 3**. Minimum Inhibitory Concentration of crude methanolic extracts of other mollusks compared *P. viridis*

# Conclusion

1. Both qualitative zoochemical screening and TLC analysis revealed that the crude methanolic extract of *P. viridis* is comprised of several zoochemicals. This include alkaloids, saponins, sterols, terpenoids, and polyphenols.

2A. Results revealed that all the test bacteria were inhibited by the extracts with *S. aureus* and *P. aeruginosa* being the most susceptible.

2B. Among the three fungi tested, *L. theobromae* was the only fungus susceptible to the extracts.

3. MIC determination showed that the extracts exhibit a promising MIC against *S. aureus* and *P. aeruginosa* with values of 2.60±0.63 and 3.65±1.69 mg/mL respectively.

# Recommendations or Future Work:

1. Further test the extracts of *P. viridis* against human pathogenic (strain) bacteria and fungi (i.e. dermatophytes)

2. Quantify the zoochemicals, particularly secondary metabolites, isolate, purify, and identify the compounds present in the extracts using more powerful instruments such as Nuclear Magnetic Resonance (NMR) Spectroscopy

3. Attempt to culture *P. viridis* in a controlled environment to maximize its potential in accumulating metabolites from its diet

4. Further test the extracts of *P. viridis* for various bioactivities and its nutraceutical potential to establish the “mussel essence”.

5. Use other mussels such as invasive “black mussel”, *Mytella charruana*.

# Acknowledgement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