Assessing Antifouling Activity of *Heteractis magnifica*

Alexandria Malilay

Big Picture

Biofouling

Current antifouling coatings

Natural antifoulants

Alternative antifoulants from *Heteractis magnifica*

Biofouling

Accumulation of undesirable organisms on immersed artificial surfaces

Coated steel grate after 9 months of exposure (left image) and uncoated steel grate after 7 months of exposure (right image) [1]

Current Antifouling Coatings

Heavy-metal ions leachate into ocean

Large contributor to marine pollution

Copper antifouling coatings banned in Holland, Sweden, and the Mediterranean

Benefits of Natural Antifoulants

Inhibit biofouling organisms while remaining non-toxic to non-target organisms and the environment

Effective at low concentrations

Biodegradable

Heteractis magnifica

Sessile organism with natural repellants

Rich in secondary bioactive metabolites

Produces nematocysts

Potential natural antifoulant

Purpose

to analyze the antifouling activity of the sea anemone Heteractis magnifica

Hypothesis

an active antifoulant of *Heteractis magnifica* will be present in the crude extract; the most effective extract will be the acetone crude extract

Materials

Amphibalanus amphitrite

Heteractis magnifica

Acetone

Ethanol

Methanol

Tissue homogenizing glass tube

DMSO

CuSO4

E. coli

Biospec Tissue Tearor

24-well & 96-well polystyrene multiwell plates

0.4% crystal violet

PBS

Incubator

Multiskan™ FC Microplate Reader

Methods: Crude Extract

H. magnifica

Biospec Tissue Tearor

Tissue homogenizing glass tube

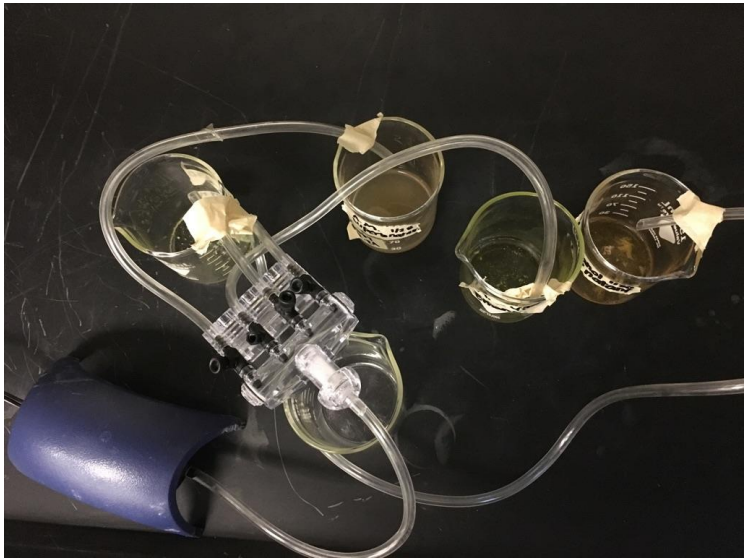
Acetone, ethanol, methanol

Centrifuge at 13 700 rpm

Vortex

Pipette

Air pump



Methods: Larval Barnacle Assay

Biopette pipettor

H. magnifica

Amphibalanus amphitrite

24-well multiwell plates

CuSO4

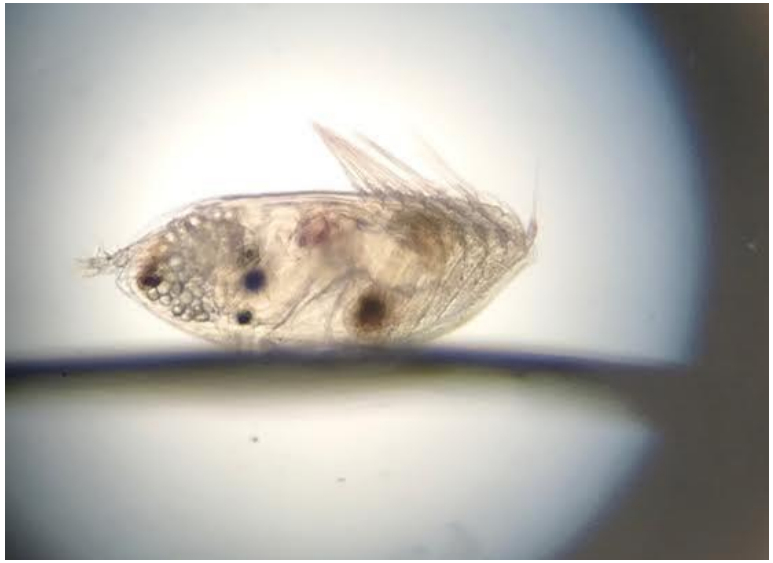
DMSO

Crude extract

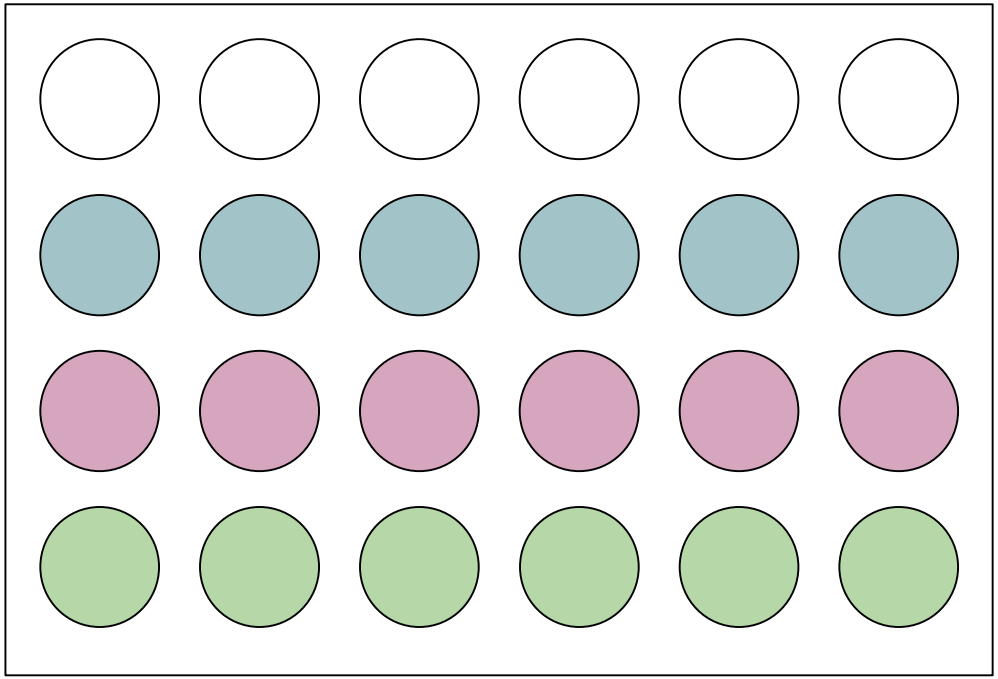
Aged sea water

Dissecting microscope

Incubator



Methods: Plate Layout



Aged sea water

CuSO4

DMSO

Crude Extract

Methods: Biofilm Assay

96-well microtiter plate

Acetone crude extract

E. coli

LB

0.4% crystal violet

DMSO

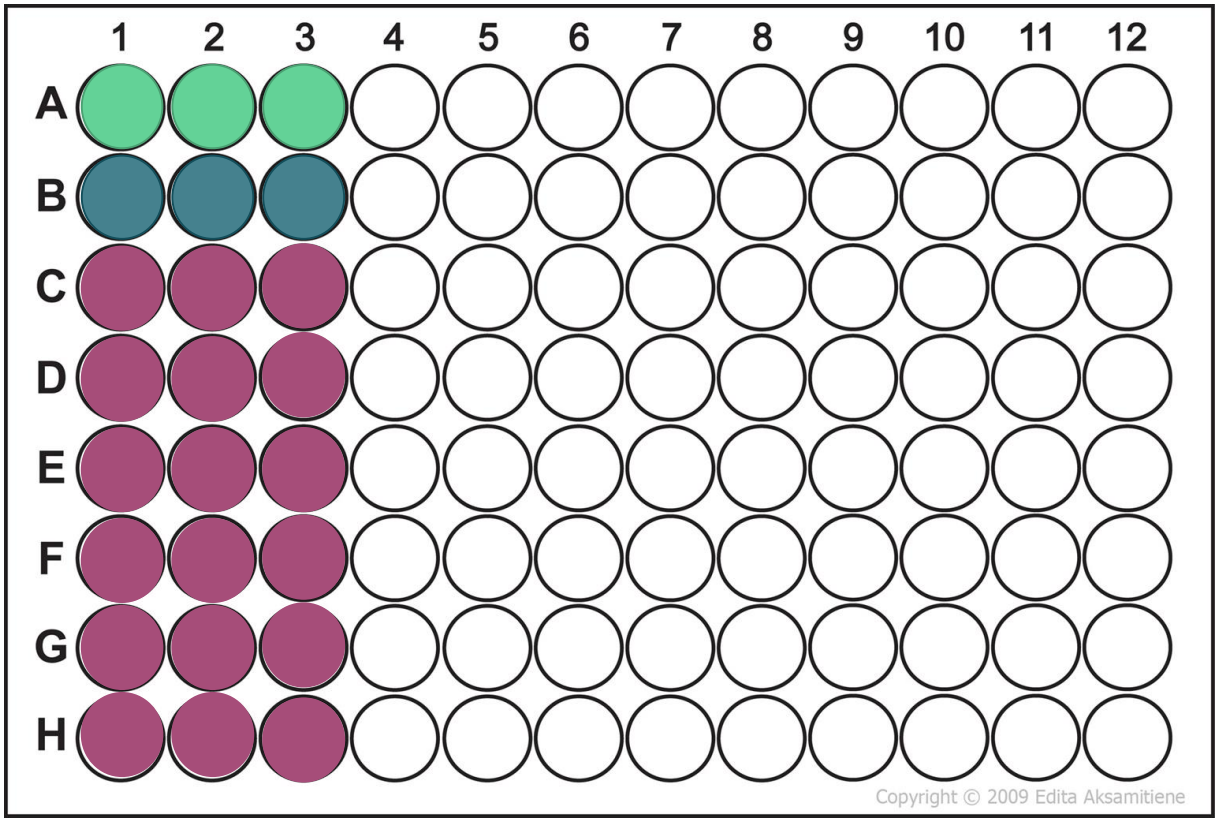
DI water

Multichannel pipettor

Buffer tray

Multiskan™ FC Microplate Reader

Methods: Plate Layout



Blank

LB

LB & *E. coli*

Varying volumes of crude extract

Results

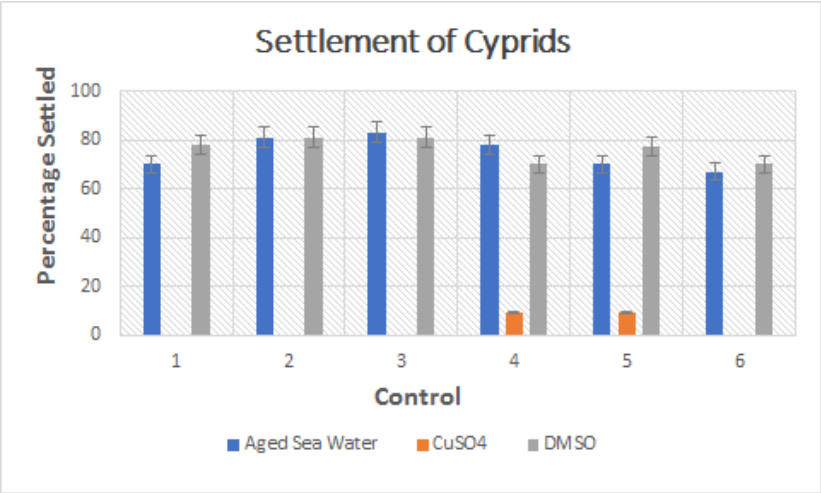


Figure 1. Represents the amount of settled cyprids after 24 h exposure to controls at 28 C.

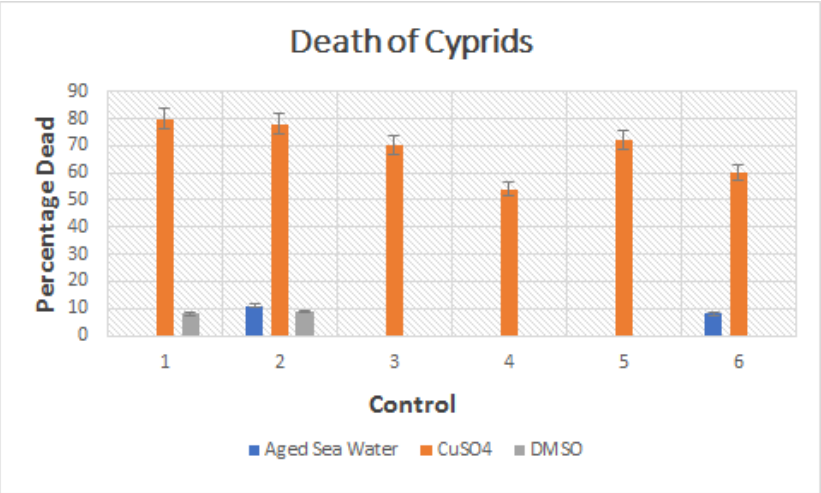


Figure 2. Represents the amount of dead cyprids after 24 h exposure to controls at 28 C.

Results

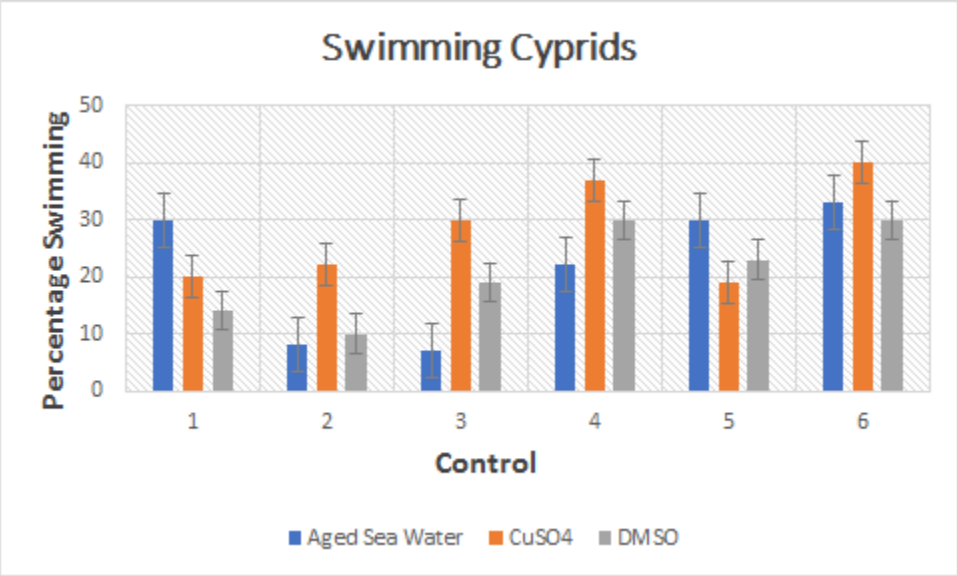


Figure 3. Represents the amount of swimming cyprids after 24 h exposure to controls at 28 C.

Results

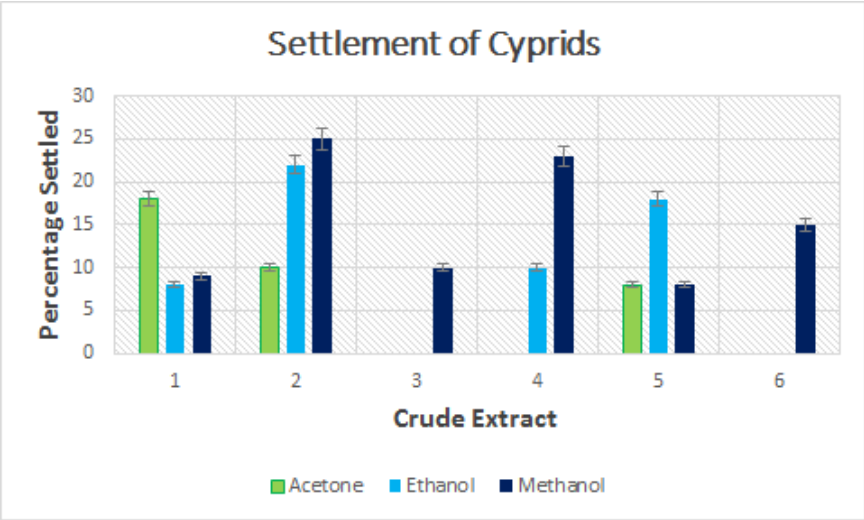


Figure 4. Represents the amount settled cyprids after 24 h exposure to crude extract at 28 C.

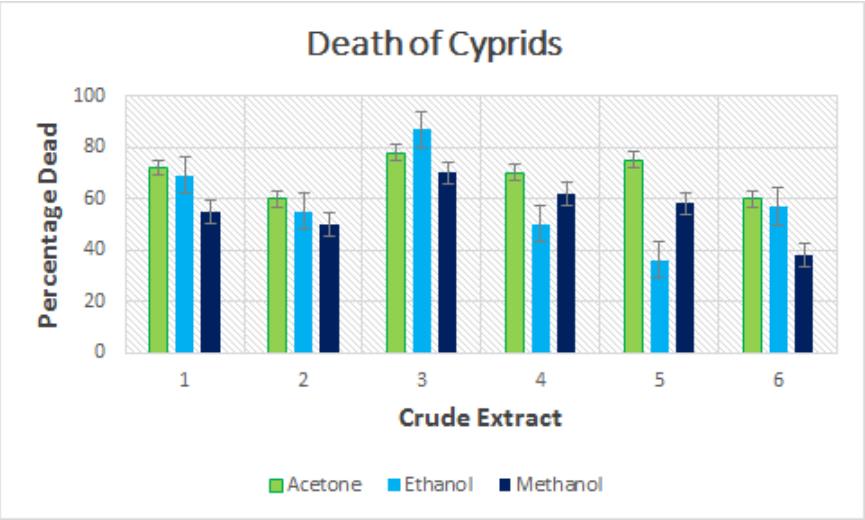


Figure 6. Represents the amount dead cyprids after 24 h exposure to crude extract at 28 C.

Results

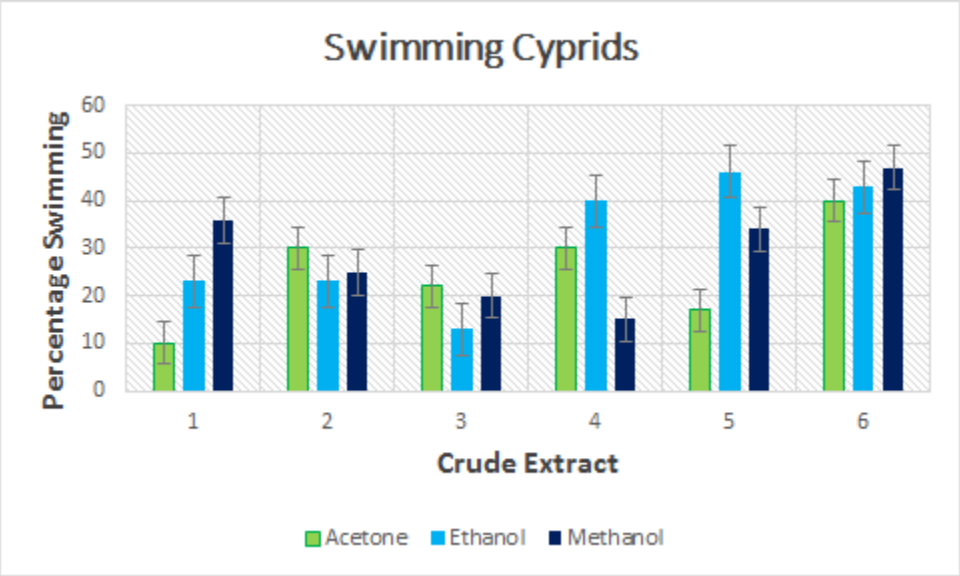


Figure 5. Represents the amount swimming cyprids after 24 h exposure to crude extract at 28 C.

Results

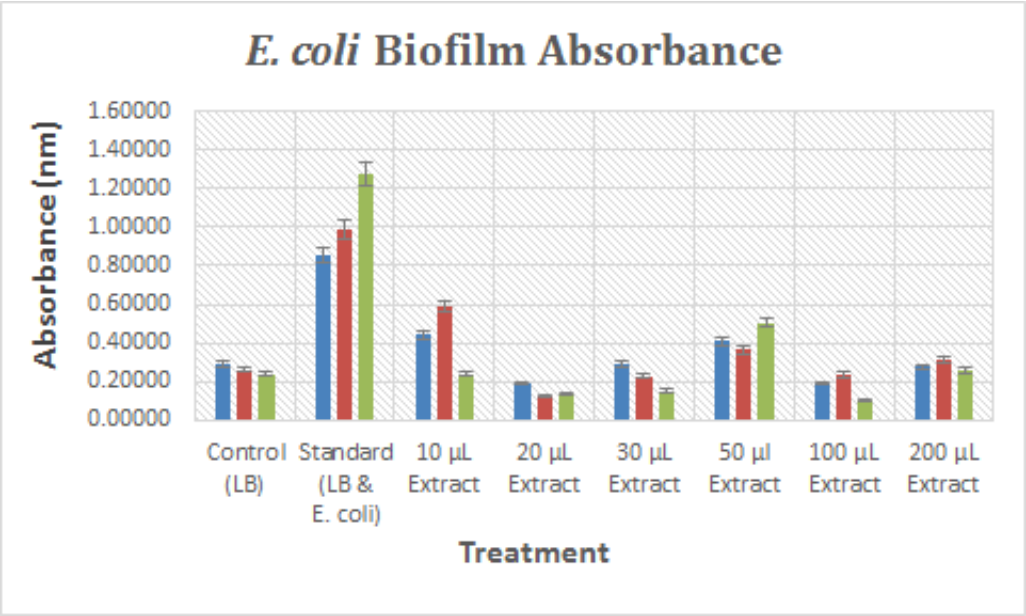


Figure 7. Represents the absorbance of the crystallized biofilm after 24 h exposure to control, standard, and acetone crude extract at 37 C.

Discussion

Acetone crude extract most effective in larval barnacle assay compared to methanol and ethanol extracts

Death rate of the cyprids for the acetone crude extract is similar to that of $CuSO_4$

Discussion

Acetone crude extract inhibited the growth of *E. coli*

20 µl impaired the most formation of biofilm

Conclusion

Both anti-macrofouling and anti-microfouling agents present

Potential antifouling agent to be extracted from acetone crude extract of *H. magnifica*

Further Work

Identify active fraction through HPLC

Conduct similar assays with purified fraction

Sources of Error

Crude extract centrifuged at room temperature rather than 4 C

Crude extract evaporated for 48 h opposed to using Rotavapor with reduced pressure

Acknowledgements

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Literature Cited/References

1. Strategic Industrial Technology Incorporated. (2013). Biofouling protection. *Strategetic*. Retrieved from http://www.strategictech.ca/BioFouling.html.

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