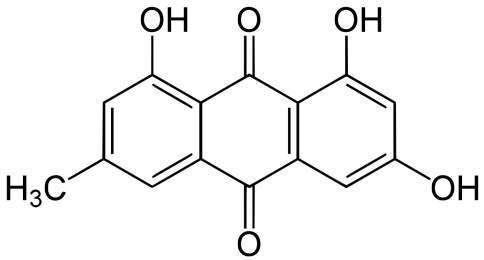
Shining a Light on Drug-Resistant Bacteria

Exploiting the Antimicrobial Effects of Anthraquinones and Reactive Oxygen Species

THE UNIVERSITY OF TEXAS AT ARLINGTON



CHEM 4392
Research Experience in Drug Discovery

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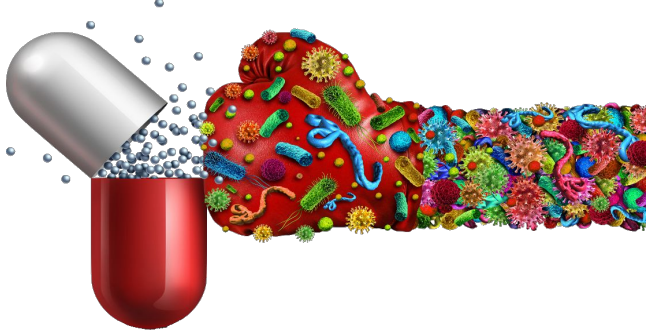
ABSTRACT



Drug–resistant bacteria and viruses are one of the most dangerous developments in recent medical history. Antibiotics have saved over 3,000,000 lives in the past 15 years, but as pathogens evolve, they become immune to many of our most effective medicines – threatening the lives of millions around the world. Our project aims to fight back, providing effective and localized treatment of infections and illness, without the possibility of developing bacterial resistance due to our unique mechanism of action. We believe our light-based treatment, which utilizes natural products to produce reactive oxygen species, will eliminate bacteria with high potency. We discovered that Japanese knotweed, rhubarb root, and yellow dock extracts are extremely promising, out of the panel of plants we have investigated so far. Our study has already produced a pipeline of natural product photosensitizers and given us a starting point to develop stronger and more effective medicines against antibiotic-resistant bacteria. Potential treatments for diabetic patients, gum disease, and many others are being considered. The bacteria found in diabetic foot ulcers in particular have shown an exceptional weakness to our light-based treatment.

https://dash.harvard.edu/bitstream/handle/1/8889467/Gottfried05.html (stats) https://www.foodsafetynews.com/2022/04/eu-figures-show-high-levels-of-antibiotic-resistance-in-foodborne-bacteria/ (image)

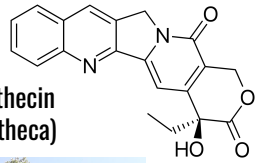
NATURAL PRODUCTS IN DRUG DISCOVERY

A myriad of life-saving medicines have been discovered and created from natural sources, such as plants and fungi. The isolation, characterization, and biological evaluation of these chemicals are what we call

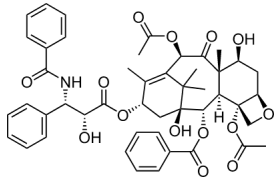
“Natural Products Chemistry”

Examples –

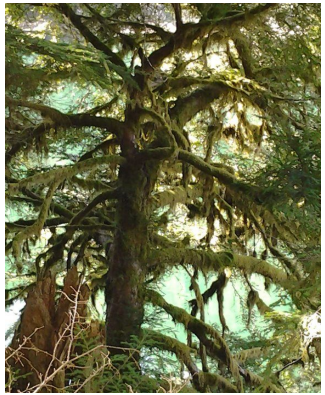
Cancer Therapeutics



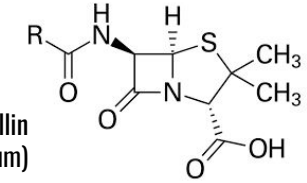
Camptothecin
(Camptotheca)



Taxol
(Pacific Yew)



Antibiotics



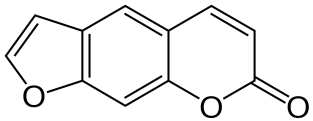
Penicillin
(Penicillium)

https://www.treesandshrubsonline.org/articles/camptotheca/camptotheca-acuminata/
http://nativeplantspnw.com/pacific-yew-taxus-brevifolia/
https://www.scientificamerican.com/article/penicillium-fungus-hosts-surprising-opioid-compounds/
https://www.dreamstime.com/flask-exotic-plant-white-organic-chemistry-flask-exotic-plant-isolated-white-organic-chemistry-image142558512

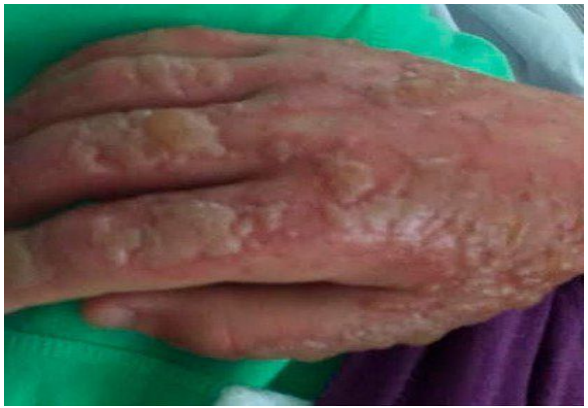
LIGHT-TRIGGERED NATURAL PRODUCTS

Giant Hogweed

Psoralen



Some plants make secondary metabolites that can be activated with light as part of a clever defense strategy. These natural product photosensitizers absorb light to facilitate photochemical reactions that are toxic to predators. For example, Giant Hogweed produces psoralen, a molecule that inflicts painful lesions on human skin under sunlight.

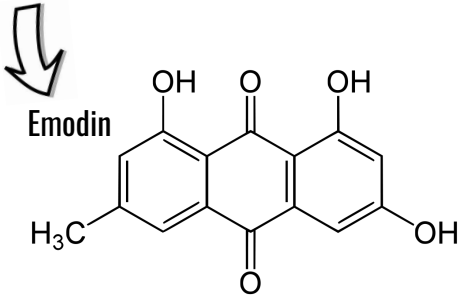


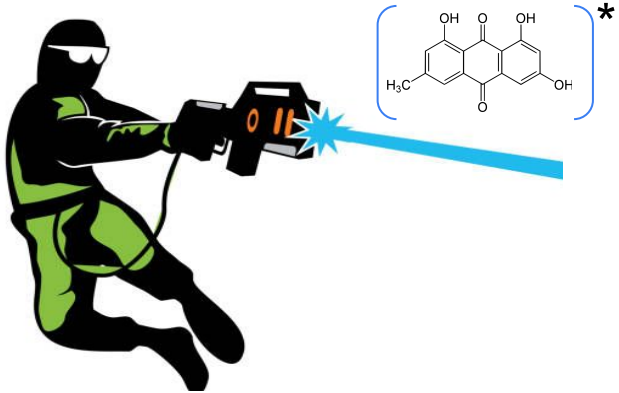
https://www.nytimes.com/2018/07/02/us/giant-hogweed-nyt.html https://www.britannica.com/plant/hogweed https://www.researchgate.net/figure/Overview-of-PDT-Following-photosensitizer-administration-it-undergoes-systemic_fig1_215563030

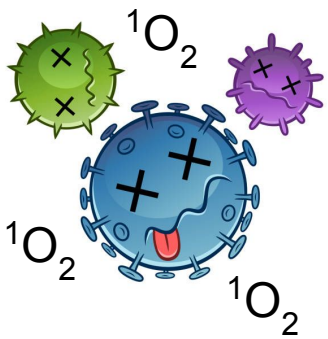
PHOTO-ANTIMICROBIALS

Japanese knotweed root

Emodin is a photosensitizer that has antimicrobial properties when exposed to light. Photon absorption produces an excited state that creates deadly reactive oxygen species (ROS) that can wipe out bacteria upon contact. Singlet oxygen ($^1O_2$) is the most important ROS with the capacity to combat drug-resistant bacteria and viruses.





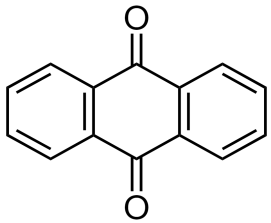


https://www.istockphoto.com/vector/laser-tag-game-player-design-illustration-gm1321923608-408034231 https://www.istockphoto.com/vector/dead-viruses-gm938431206-256623071
https://firstaidtrainingcooperative.co.uk/treatment-following-contact-with-giant-hogweed https://en.wikipedia.org/wiki/Singlet_oxygen

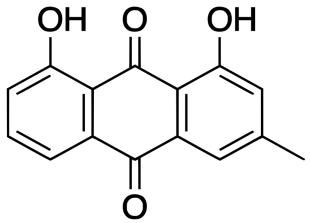
ANTHRAQUINONES

Extracts from plants such as Japanese knotweed *(P. cuspidatum)*, rhubarb *(R. hybridum)*, and yellow dock *(R. crispus)* have been reported to exhibit antimicrobial activity. They also contain various anthraquinones, which are known to have photosensitizing properties.

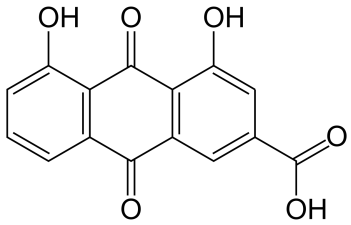
Anthraquinone



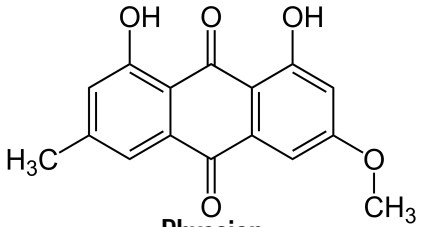
Naturally occurring anthraquinone derivatives



Chrysophanol



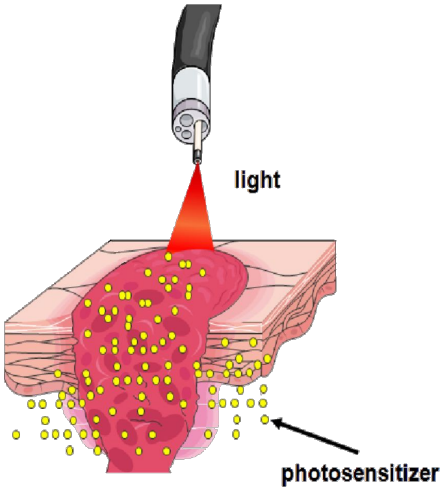
Rhein



Physcion

HYPOTHESIS / SCIENTIFIC PREMISE

We believe that by utilizing the antimicrobial properties of emodin and other anthraquinones, we can counter the epidemic of drug-resistant pathogens. Our light-based treatment overwhelms pathogens with ROS, preventing them from evolving further immunity. There are a wide variety of natural products that contain these photosensitizers, and we have produced a pipeline to analyze and extract from these sources.



There are many potential applications of this treatment, and our research has given us a great starting point to further develop stronger and more effective defenses in our fight against drug-resistant bacteria.

https://herbies-herbs.com/japanese-knotweed-p1024

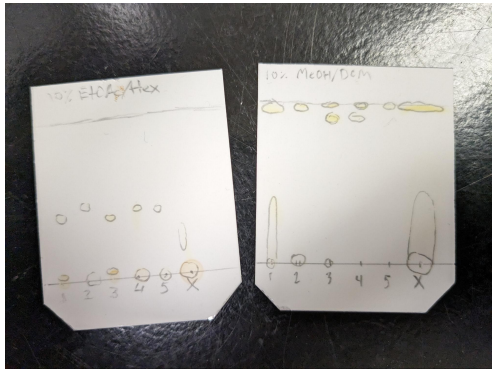
APPROACH & WORKFLOW

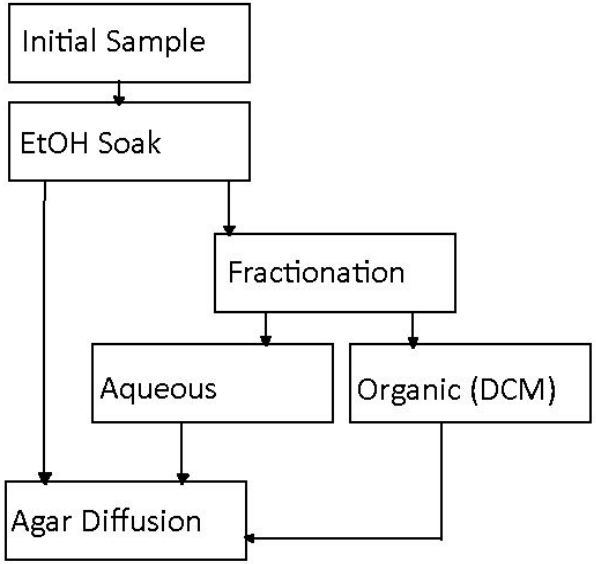
Goal: compare the photo-antimicrobial effects of root extracts that contain various photosensitizing anthraquinones

yellow dock
R. crispus

Japanese knotweed
P. cuspidatum

rhubarb
R. hybridum





Extracts are obtained using different extraction methods and fractionation techniques for comparison of their photo-antimicrobial activities using a high-throughput agar diffusion assay against *S. aureus*. The results are being used for further bioassay-guided fractionation toward optimization of activity and identification of bioactive photosensitizers.

WHOLE EXTRACTS FROM ETHANOL

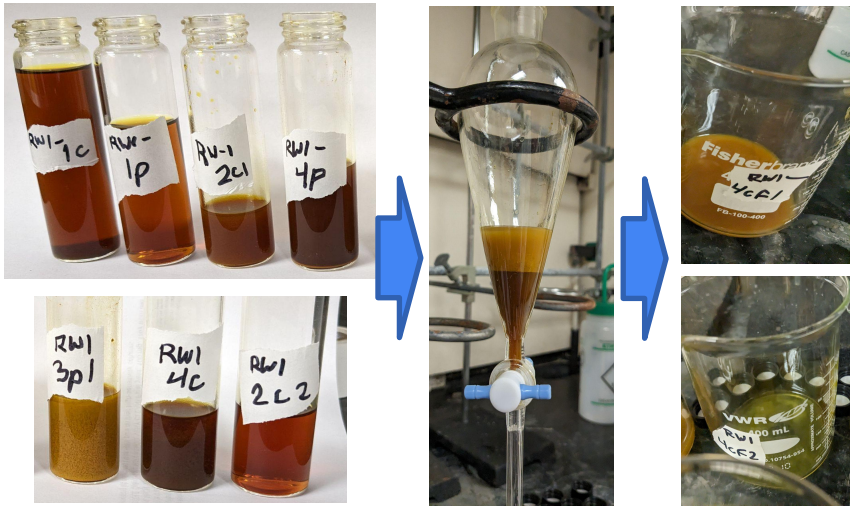
Entry	Material	Code	Supplier
1	Yellow Dock Powder	RW1-6Ca	Organic Way
2	Yellow Dock Chips	RW1-6Pa	Bixa Botanical
3	Japanese Knotweed Powder	RW1-Pb	Nature Restore
4	Japanese Knotweed Chips	RW1-Cb1	Thera-Plants
5	Japanese Knotweed Chips	RW1-Cb2	Nuherbs Organics
6	Rhubarb Root Powder	RW1-Pc	Starwest Botanical
7	Rhubarb Root Chips	RW1-Cc	Starwest Botanical

5 g of each sample was extracted with 200 mL of ethanol at room temperature for 24 h. The ethanol solutions were filtered and left open to air to evaporate at room temperature.

FRACTIONATION OF ETHANOL EXTRACTS

Entry	Material	Code	Supplier
1	Yellow Dock Powder	RW1-1P	Bixa Botanical
2	Yellow Dock Chips	RW1-1C	Organic Way
3	Japanese Knotweed Powder	RW1-3P1	Nature Restore
4	Japanese Knotweed Chips	RW1-2C1	Nuherbs Organics
5	Japanese Knotweed Chips	RW1-2C2	Thera-Plants
6	Rhubarb Root Powder	RW1-4P	Starwest Botanical
7	Rhubarb Root Chips	RW1-4C	Starwest Botanical

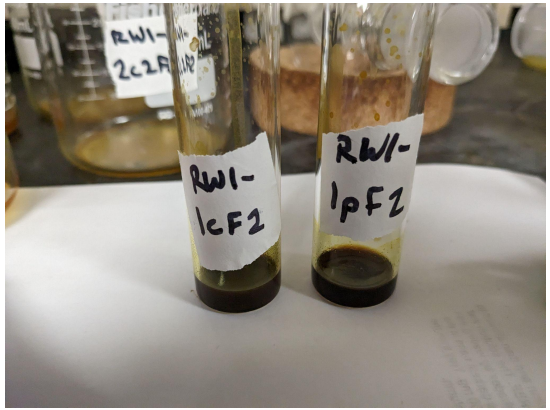
Another set of ethanol extracts was prepared as described and partitioned between water and dichloromethane (DCM) to yield aqueous (f1) and organic (f2) fractions.



Yellow Dock		
Code	Fraction	% Recovery
RW1-1pf1	Aqueous	0.72%
RW1-1pf2	Organic	0.84%
RW1-1cf1	Aqueous	5.13%
RW1-1cf2	Organic	1.50%
RW1-6ca	Ethanol	6.28%
RW1-6pa	Ethanol	2.78%

Japanese Knotweed		
Code	Fraction	% Recovery
RW1-2c1f1	Aqueous	3.25%
RW1-2c1f2	Organic	0.30%
RW1-2c2f1	Aqueous	2.06%
RW1-2c2f2	Organic	0.20%
RW1-3p1f1	Aqueous	X
RW1-3p1f2	Organic	1.57%
RW1-6cb1	Ethanol	X
RW1-6cb2	Ethanol	13.51%
RW1-6pb	Ethanol	4.91%

Rhubarb		
Code	Fraction	% Recovery
RW1-4cf1	Aqueous	2.69%
RW1-4cf2	Organic	0.20%
RW1-4pf1	Aqueous	4.96%
RW1-4pf2	Organic	0.48%
RW1-6cc	Ethanol	X
RW1-6pc	Ethanol	X



~5g of plant material was soaked in 200 mL EtOH for each extraction.

% RECOVERY FROM BIOMASS

SOP FOR ANTIMICROBIAL ACTIVITY

- Extracts were prepared as stock solutions in 100% DMSO
- 20 µL of 5 mg mL^{-1} stock solution was tested against *S. aureus* culture at 2×10^8 CFU mL^{-1} for an incubation time of 24 h
- Control experiments determined that extracts were inactive without a light trigger (see below)
- Light treatment: broadband visible light delivered at a fluence of 100 J cm^{-2} and irradiance of 27.3–29.2 mW cm^{-2}
- All the extracts showed photo-antimicrobial activity as inhibition zones (next slide)

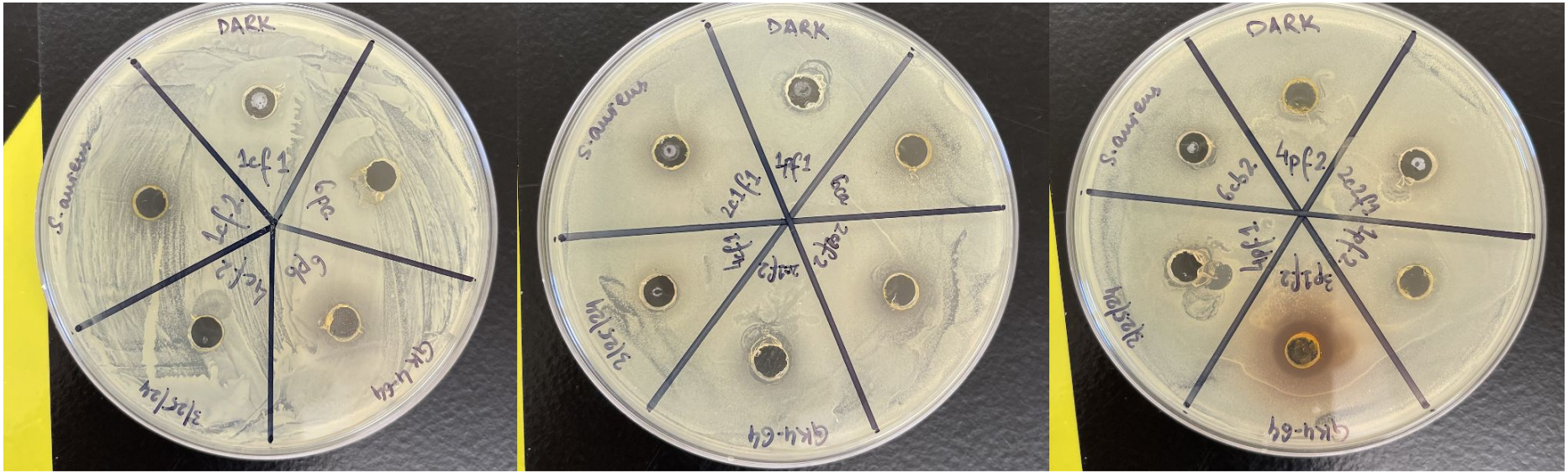
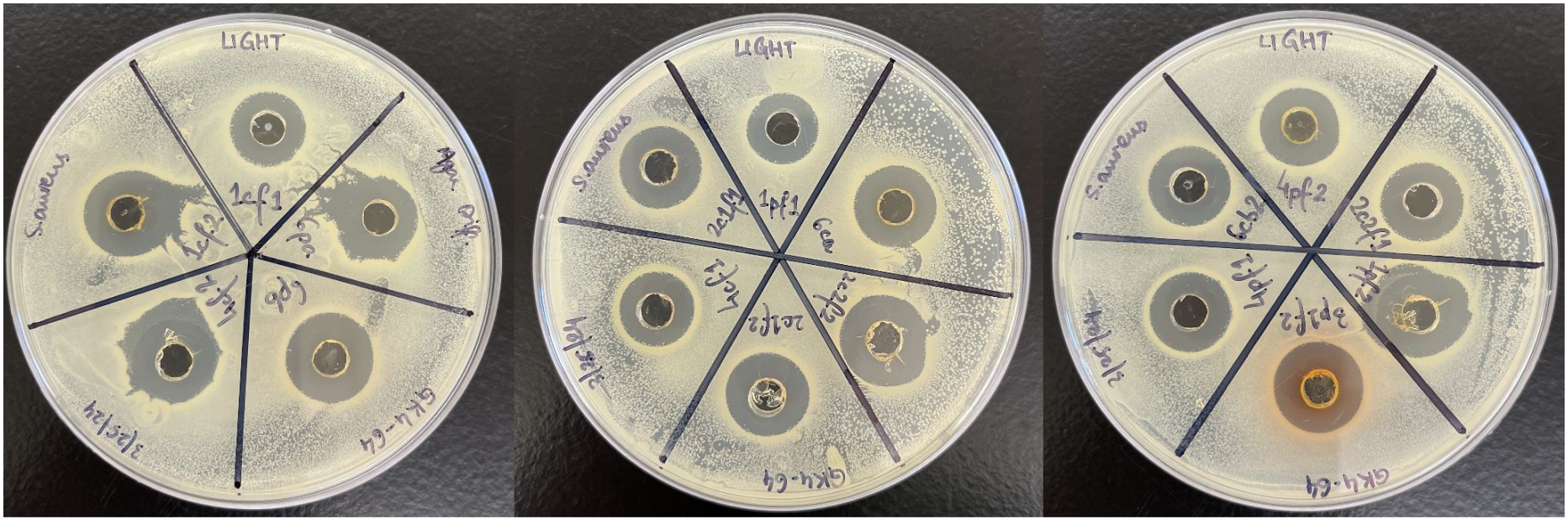


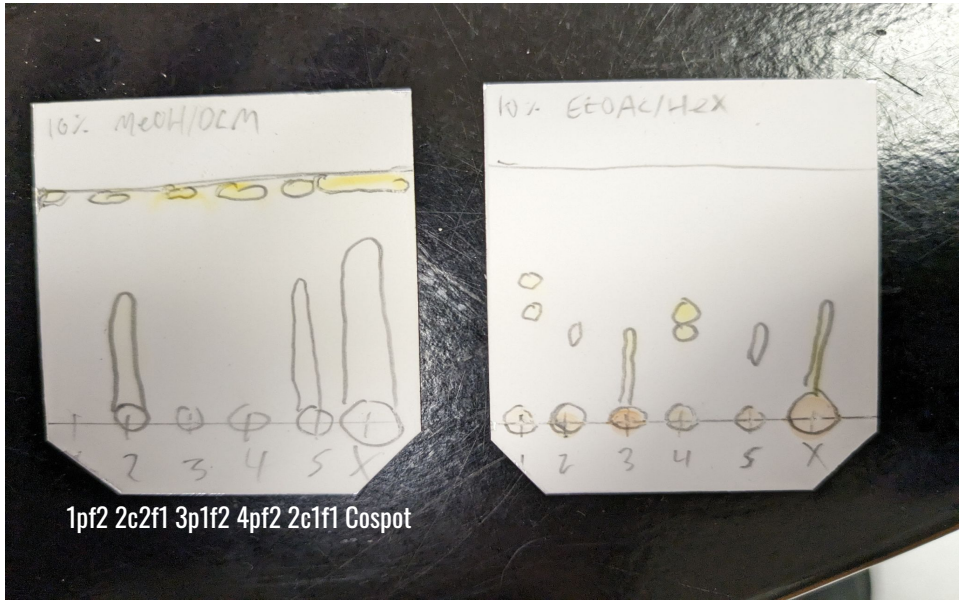
PHOTO-ANTIMICROBIAL ACTIVITY

	Inhibition (cm)		Inhibition (cm)		Inhibition (cm)
RW1-1pf1	1.3	RW1-2c1f1	1.5	RW1-6pb	1.6
RW1-1pf2	1.7	RW1-2c1f2	1.5	RW1-4cf1	1.3
RW1-1cf1	1.4	RW1-2c2f1	1.45	RW1-4cf2	1.65
RW1-1cf2	1.5	RW1-2c2f2	1.65	RW1-4pf1	1.5
RW1-6ca	1.5	RW1-3p1f2	1.6	RW1-4pf2	1.4
RW1-6pa	1.5	RW1-6cb2	1.5		



SUMMARY OF RESULTS & CONCLUSION

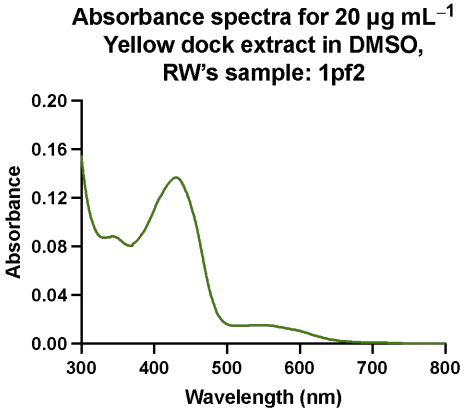
Japanese knotweed, rhubarb, and yellow dock root extracts all show strong inhibition zones. DCM fractions generally show stronger inhibition than aqueous fractions, and are potentially stronger than simple ethanol soaks. More tests on this are prepared for the future.

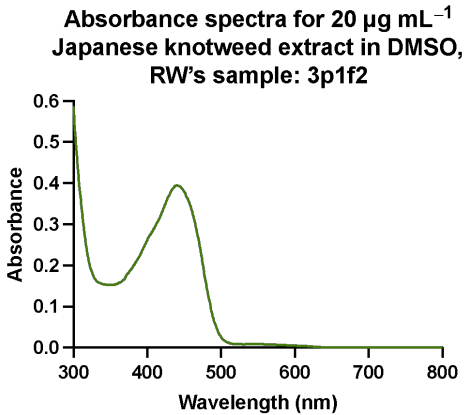


1. DCM Yellow Dock
2. Aqueous J. Knotweed
3. DCM J. Knotweed
4. DCM Rhubarb
5. Aqueous J. Knotweed

We conducted a series of TLCs. 10% MeOH in DCM shows very strong mobility in all samples compared to EtOAc in Hexane. We are also prepared to conduct more TLCs with different concentrations and solvents to determine the chromatography methods we will use toward bioassay-guided fractionation.

FUTURE STUDIES



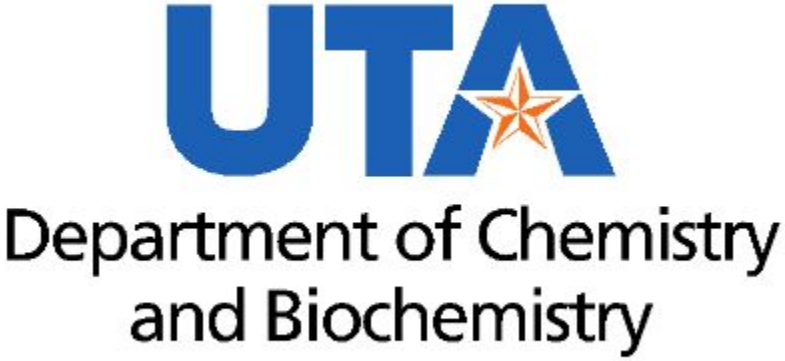


Absorption profiles of the extracts are being analyzed to determine the optimal wavelengths to use in subsequent photo-antimicrobial assays toward optimization of the activity. Yellow dock extract (left) could be activated by blue, green, or red light. Japanese knotweed extract (right) is likely best activated by blue light.

A previous study (doi 10.1055/a-1652-1547) reports on the antimicrobial properties of yellow dock, but did not investigate its photo-antimicrobial properties. Further our studies show no antimicrobial activity in the dark which contrasts with the published study. We aim to carry out a systematic investigation for comparison and follow-up to that article.

We aim to identify the active photosensitizers in these extracts using bioassay-guided fractionation. Once known, the extraction methods, fractionation steps, and purification techniques can be optimized for enriching the extracts in the most potent photosensitizer(s). These optimized extracts will be studied spectroscopically and biologically.

ACKNOWLEDGEMENTS



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