How can local plants in the Jawoyn region be used to prevent bacterial infections?

Report by Tahnee Brown
Submission supported by teacher, Genevieve Firmer

Scientific Report Prepared in 2019 for Year 11 Biology
Deconstruct and Design Task

Author Profile by Tahnee Brown

My name is Tahnee Brown, and I am an Aboriginal woman from Katherine which is in the Jawoyn Region. My family is originally from New South Wales, but my grandfather moved to the Top End for work when he was younger.

The topic of my assignment was to see the antibacterial properties of traditional Indigenous medicinal therapies. I chose to base the assignment on investigating this topic to better my understanding of the ways in which Indigenous people relieved sicknesses before having the ability to access western medicines.

In order to gather this information, a variety of different approaches were used. Books and websites were used to gather the information regarding the plants located in the Katherine region with medicinal properties. To gather the steps of preparation of the plants, a traditional owner was interviewed. Ozzie Daylight, interviewed, is the traditional owner of Elsey Station. This is located about fifteen kilometres out of Mataranka. I know Ozzie through Dad, Dad has been friends with many of the traditional owners in this area for many years. He explained the ways in which the plants were prepared and used and depending on the type of sickness, which plants are to be used. With this information the plants were prepared the way that the Indigenous peoples of the Northern Territory prepared them in the past. These steps of preparation are still used today by many Indigenous peoples, but is more commonly associated with medicine preparation in the past.

At the start of the project I was working with another student, whose family is from Millingimbi, and this made talking about the topic interesting as some of the ideas and practices conflicted. Before the preparation of these plants, a plan was made which came over the conflicts and therefore, a viable experiment was agreed upon.

In saying this, the project was quite enjoyable as it was a bit different to other classroom projects. Learning antibacterial properties of generalised plants is one step to better understand Indigenous cultures. I believe that other projects that investigate not only Indigenous cultures but many cultures should be completed in schools due to the fact that Australia is a multicultural continent.

I am interested in learning how medicines and other substances effect the brain and body. This includes the produce of diagnosing and treating patients. I am interested in what could be used as a better alternative to western medicines.

In the future I plan to continue studying the effects that different medicines have on the body and how we can use first nations practices into the recognised medical fields.

Photo: Entrance to Kakadu via Manyallaluk, taken in 2019. From the left: Tahnee Brown, Genevieve Firmer. Manyallaluk in a small community situated roughly one hundred kilometres out of Katherine. My Dad has been going camping there before I was born, and I have been there since I was a little.

Teaching Notes by Genevieve Firmer

For this task, students were given free choice to design and conduct a scientific investigation related to our topic of microorganisms. The best thing about being a teacher is working with great students, particularly when they bring fresh, creative and innovative ideas about solving complex problems into the classroom. When the idea about investigating something to do with local Indigenous knowledge came up in discussions, Tahnee enthusiastically took on the challenge. She consulted a book we had in the classroom about medicinal plants in the region and started a conversation with Ozzie Daylight, a traditional owner of the nearby Elsey Station, whom she has known through her family connections for many years. Tahnee brought all this information together to design the scientific investigation presented below about the anti-bacterial properties of a selection of local plants.

Infectious diseases are increasingly becoming a burden on society and the exploration of natural products for new potential drug molecules is more important than ever. Tahnee's project is a beautiful example of how students with diverse backgrounds can help to unlock information about natural products which may hold the key for scientific breakthroughs. Tahnee has used the strength of her family connections to navigate the process of community engagement in a way that is responsive to traditional owners and community.

This work is also a demonstration of how secondary students can use the existing frameworks for year 11-12 scientific investigation assessments to utilise contemporary scientific practices in the investigation of Indigenous knowledges. These skills are critical for progress to be made in the genuine engagement of First Nations peoples in scientific research, and projects like this allow students to see themselves and their strengths reflected in the curriculum.

This piece has been presented exactly as it was submitted it for a year 11 biology assignment as an authentic example of the work that students can achieve in a school environment. Permission has been granted by Tahnee and her family to share this work. Small sections of information have been withheld to protect the traditional knowledge shared with the student in confidence.

I would like to thank Ozzie for his willingness to work with Tahnee on this project and thank Tahnee and her family for agreeing to share this work publicly. Thanks also to Jesse King for the support in readying this piece for submission.

I would like to acknowledge the Jawoyn, Dagoman, Wardaman and Miali people, the traditional owners and custodians of the land on which this work was conducted, and where the authors lived at the time this project was completed.

How can local plants in the Jawoyn region be used to prevent bacterial infections?

By Tahnee Brown

Introduction

Medicinal plants are plants that have healing properties or use beneficial pharmacological effects in a living organism. These types of plants have been used as traditional medicines in many cultures around the world. Therefore, the history of medicinal plants is just as long as the history of humans.

Figure 1 - Red Bush Apple

Medicinal plants have played an essential part as sources of drug lead compounds of Indigenous peoples in the Northern Territory lives, to decrease bacteria inflections in their community. The knowledge of the plant's application, location and structure was passed down through generations via song and dance. Indigenous peoples remembered the ideal season for selection and the correct method of preparation and indicators for use. This gained knowledge is vast and critical for survival.

Figure 2 - Gumbi Gumbi Bush

These medications where either applied internally or externally to relieve pain, promote healing, or cursing such elements that was common to them. For this reason, there does not appear to be any traditional medicines that can cure or decrease the bacterial infections for those diseases introduced by colonising races.

Indigenous people confine their use to plants within their respective areas and do not consider them as having therapeutic value outside this area. The same plant that grows in two tribal areas is often used in very different ways medicinally. Different parts of the plant are considered by one tribe to be more important than another or vary in preparation or use indication.

Aim

To determine which Jawoyn plants are optimal to decrease the rate of bacterial infections.

Hypothesis

If plant extracts are added to the bacteria by having both, bacteria and plant extract, on the same agar plate the growth rate of bacteria will decrease because plants have bacteria killing antibodies.

Variables

Independent Variable: Solution placed on agar plate.
Dependent Variable: Bacteria Growth
Uncontrolled Variable: Amount of Agar in Plates, how tight the dish is sealed, temperature in cupboard.
Controlled Variable:

Table 1 – Controlled Variables

Variable	Why it needs to be controlled	How will it be controlled
Amount of water	The same amount of water needs to be controlled because then the concentration of the plant is the same throughout all of the experiment.	50mL
Amount of Plant	The amount of plant needs to be kept the same so then the concentration of the plant to water is the same throughout the entire experiment.	1g
Size of container	The size of the container needs to be the same to have the same amount of bacteria on the agar place at the start of the experiment.	Petri Dish
Amount of nutrients	This has to be controlled so that each bacteria has the same living conditions.	The same nutrients and amount will be used
Temperature	The temperature that the bacteria are growing all the same, in case the bacteria grow faster or slower in different temperatures.	They are stored in the same area
Human Interaction with Agar Plates	If there is human interaction with the agar plates than the bacteria will start to grow, and the experiment will not be valid.	Do not breath or touch the agar plates

Safety

Table 2 - Safety

Hazard	Risk Level	Precautions
Broken Glass	Low	- Stir everything cautiously and gently - Immediately notify supervising adult
Split water	Low	- Place beakers away from the edge of the table - Clean up any spills immediately
Burn from Fire	Low	- Do not touch or play with fire - Pay attention when in use - Turn off when not in use
Risk of Bacterial Infection	Medium	- Do not incubate bacteria at body temperature - Don't infect petri dish with human bacteria - Don't close the plates entirely - Do not reopen plates

Materials

- 5x Bark and Leaf samples
- 5x 250mL Beakers
- 2x Bunsen burner
- Cotton Swabs approx. 10
- 2x Gauze Mat
- 2x Heat Mat
- 5x Mortar and Pestle
- 50mL Measuring Cylinder
- 12x Petri Dish
- 1x Permanent Marker
- Personal Protective Equipment (PPE)
- Scales
- Scissors
- Tape
- Timer
- Tongs
- 2x Tripod Stand
- Water approx. 500mL

Method

1. Collect five different leaf and bark samples and take photos of the different trees for evidence and classification.
2. Separate the samples. Using separate mortar and pestles crush up the plants as much as you can. (Figure. 2)
3. Place the crushed mixture into 250mL beakers, and using a permanent marker label the beaker with the plant used.
4. Prepare five beakers with the following:
    - 1g of crushed plant sample
    - 50mL of water
5. Prepare the control beaker with the following:
    - 50mL of water
6. Setup two Bunsen burners; Heat Mat, Tripod Stand, Gauze Mat, Bunsen Burner, Tongs and PPE. Boil the plant mixtures for five minutes each. (Figure. 3)
7. After all of the mixtures have been boiled, get a new 250mL beaker and fill will with 50mL of water.
8. Dampen a new cotton swab with the water from new beaker and collect bacteria from hands and bench.
9. Then contaminate the petri dish with the cotton swap by swiping side to side all the way down the dish. Turn the petri dish slightly and repeat this four times and after each trial.
10. Repeat steps 8 and 9 for all of the petri dishes.
11. For each of the plant samples, label two petri dishes with a permanent marker the plant used.
12. Using a new cotton swab spread the boiled mixture over the agar plates. A different cotton swab needs to be used with a different plant mixture.
13. Repeat step 11 for all of the agar plates.
14. Once the samples have been coated in the plant mixture tape the cover to the bottom of the petri dish. (Figure. 4)
15. Place all of the petri dishes upside down in a cupboard out of human interaction to grow.
16. Check and analyse the bacteria in five days.

Figure 3 - Crushing Up the Plants

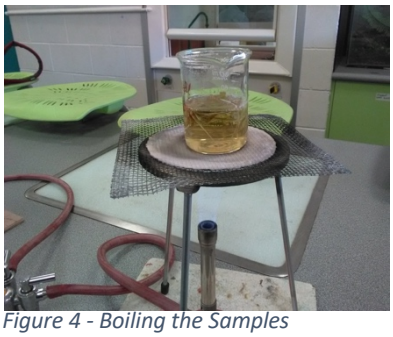


Figure 4 - Boiling the Samples

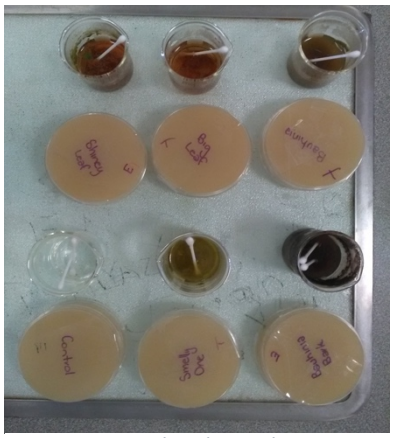


Figure 5 – Completed Samples

Results

After the experiment the results support my hypothesis as there was less bacteria in the treated petri dishes compared to the controlled treatment method.

Table 3 – Difference Between Control and Treatment

	Control	Shiny Leaf	Bauhinia Bark	Bauhinia	Big leaf	Smelly
Number of Types	3	3	3	3	4	4
Description	Yellow	Fluffy White	Red	Fluffy White	Black Fluffy	Little Fluffy
	White	Yellow	White	Yellow	White	Fluffy Black
	Fluffy black	White	Yellow	White	Yellow	White
					Little Fluffy Dots	Yellow
Fluffy Black	4	0	0	0	0	1
Fluffy White	0	1	0	1	1	0
Little Fluffy	0	0	0	0	2	1
Red	0	0	15	0	0	0
White	20	234	57	23	36	30
Yellow	24	94	38	21	7	10

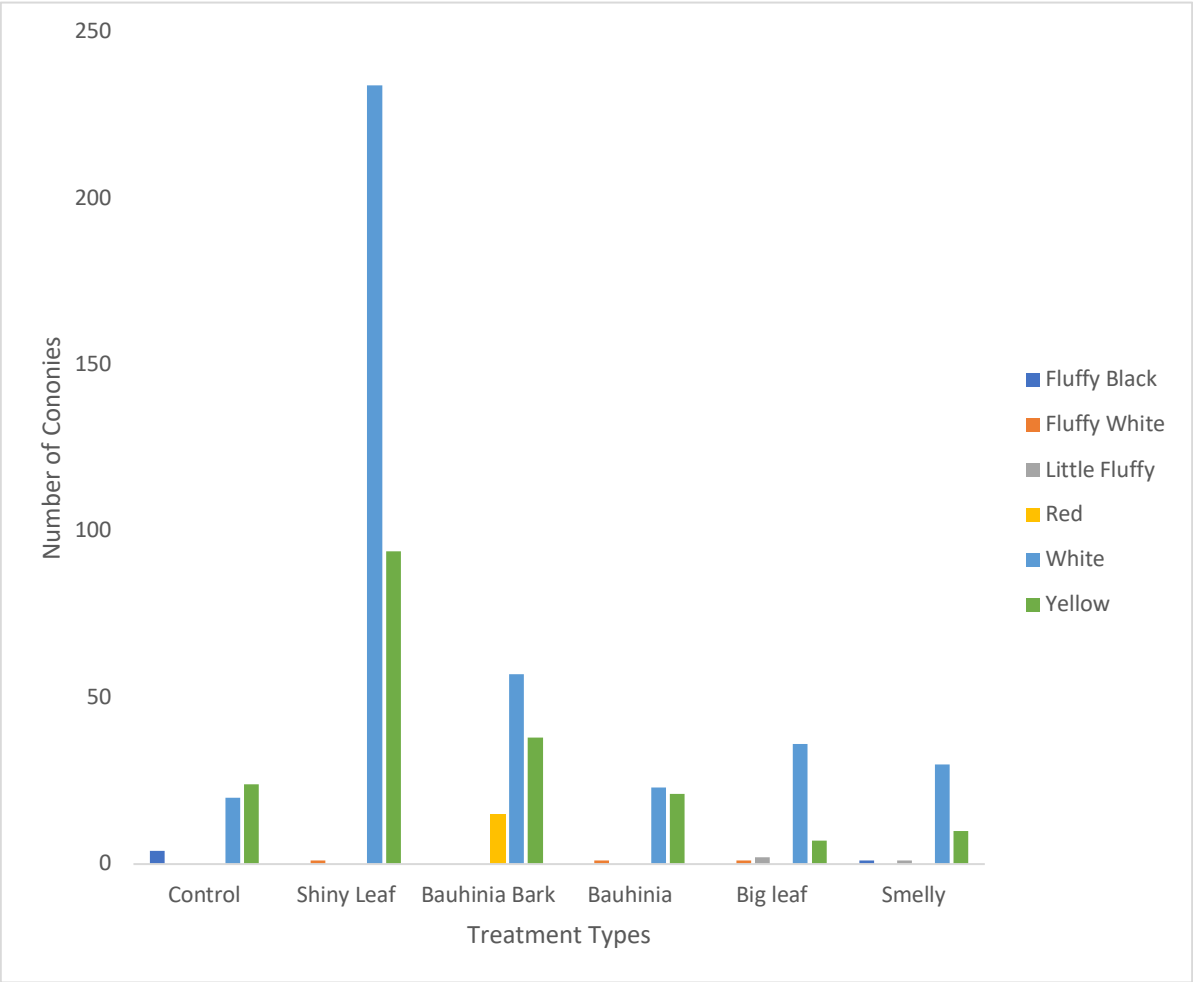


Figure 6 - Difference Between Control and Treatment

Discussion

These results support the hypothesis as there was less bacteria in some of the petri dishes compared to the control trials. The different plant samples decreased the growing rate of the bacteria collected from various surfaces for some of the trials. The control petri dishes had a total number of 48 colonies of bacteria with only three different types of colonies between the both of them. These colonies included; a fluffy black irregular shaped bacteria colony, 20 small to medium sized white colonies and 24 small to mild sized yellow bacterium colonies.

The shiny leaf did not grow a fluffy black bacteria colony like in the control petri dish but instead grew one fluffy white colony and the number of white and yellow bacteria colonies increased drastically, with 234 white bacteria colonies and 94 yellow colonies between the two petri dishes.

The treatment that worked the best for decreasing the number of colonies of bacteria is the Bauhinia leaves with three different bacteria colonies types; these where identified as yellow, white, and fluffy white bacteria colonies. There was, in total, one fluffy white bacteria colony, 23 white bacteria colonies, and 21 yellow bacteria colonies. This means that the bauhinia leaves may have antibacterial properties as they decreased the number of some of the bacteria colonies on the petri dishes. These results are backed up by the authors Gupta and Paarakh at The Oxford College of Pharmacy, as “it was found that aqueous extract has antibacterial activity”.

The bauhinia bark had a total of 110 bacteria colonies. These included 15 red colonies, 57 white colonies and 38 yellow bacterial colonies. The bauhinia was the only bark that was selected to use and the only product with red bacteria colonies on the agar plate. This shows that the bauhinia bark and the bauhinia leaves have different antibacterial properties.

Evaluation

Table 4 – Systematic and random Errors

Systematic Errors	Reasoning	Effect on Conclusions
The scales might not be precise	If the scales where not precise with weighing out the plants, there might only be 0.97g. The scale would have been accurate as it has the same weight in all of the beakers, but not precise.	This effects the conclusions by the trials may have slightly less/more antibacterial agents.
The measuring cylinders may not be precise	The measuring cylinder might not have been precise with measuring out the amount of water, there might only be 48mL instead of 50mL.	This effects the conclusions by the trials may have slightly less/more antibacterial agents.
Random Errors	Reasoning	Effect on Conclusions
Swabbing of the Agar Plate	The swabbing motion onto the agar plate was the same but the amount that actually got stuck to the agar plate might have been different.	This effects the conclusions by the trials may have slightly less/more agents compared to the other trials, this is limited by using the same swabbing technique.
Amount of Bacteria on Agar Plate	The amount of bacteria collected from the surfaces used might be different amounts in different places on the bench.	This effects the conclusions by the trials may have slightly less/more bacteria that could grow on the agar plates, to limit this the same swabbing pattern was used.
Amount of Treatment on Agar Plate	The amount of treatment that was carried on the cotton swab may have been different for all of the treatment trials.	This effects the conclusions by the trials may have slightly less/more treatment agents compared to the other trials. To limit this the same swabbing pattern was used.

Conclusion

The experiment that was undertaken supported my hypothesis that the plants will decrease the rate at which the bacteria will grow, as some of the trials have less bacteria grown compared to the control trials. The best plant for decreasing the rate of which the bacteria will grow in this experiment was the bauhinia leaves. This was made accurate by considering the variables and limited their impact on the experiment. There were still some errors including; systematic and random errors. Overall the plants decreased the growth of the bacteria.

References

- Aboriginal Pharmacopeia, *The State Reference Library of the Northern Territory,* accessed 22nd November 2019, <https://www.territorystories.nt.gov.au/jspui/bitstream/10070/153108/1/occpaper10.pdf>.
- Brought To Life, *Making Medicines,* accessed 23rd November 2019, <http://broughttolife.sciencemuseum.org.uk/broughttolife/themes/treatments/medicines>.
- Cancer Council, *Is Gumbi Gumbi effective in treating cancer?,* accessed 23rd November 2019, < https://iheard.com.au/question/is-gumbi-gumbi-effective-in-treating-cancer/>.
- Microbiology Learning, *The "Why"ology of Microbial Testing,* accessed 23rd November 2019, < https://microbiologylearning.weebly.com/streaking-agar-plates-4-quadrant-streak-method.html>.
- Science Buddies, *Tiny Titans: Can Silver Nanoparticles Neutralize E. Coli Bacteria?,* accessed 22nd November 2019, <https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p031/microbiology/can-silver-nanoparticles-neutralize-e-coli-bacteria>.
- Science Direct, *Medicinal Plants,* accessed 21st November 2019, <https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/medicinal-plant>.
- Taste of the Top End, *Our red Bush Apple- Which isn't an apple at all,* accessed 19th November 2019, <https://tasteofthetopend.com/2014/12/16/our-red-bush-apple-which-isnt-an-apple-at-all/>.
- https://www.tsijournals.com/articles/antibacterial-activity-of-different-extracts-of-the-plant-bauhinia-variegata.pdf

Appendix A – Investigation Planning

What Affects the Growth of Bacteria?

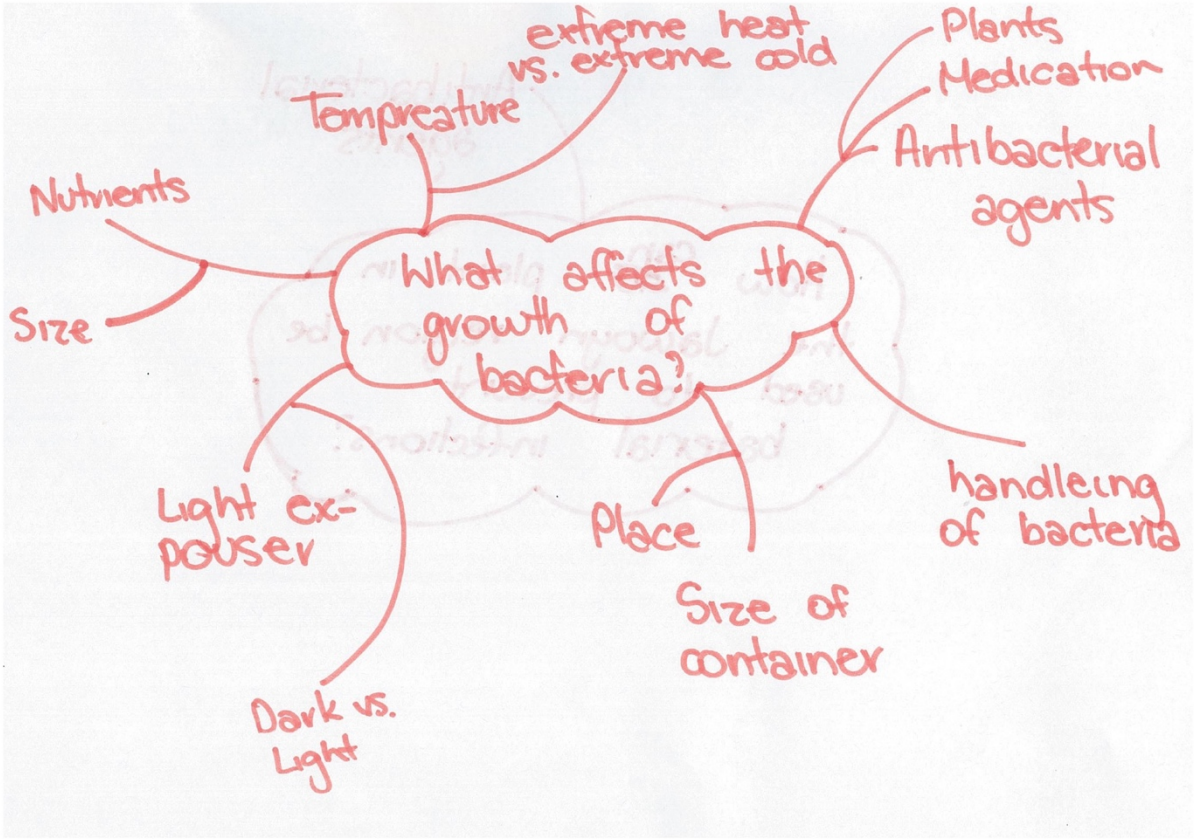


Figure 1 - What Affects the Growth of Bacteria?

Ways to measure the product

There are multiple ways to measure the outcome of the bacteria growth/death. This includes the size of bacterium, amount of bacterium, number of different types of bacterium, weight, and percentage of coverage. These measurements would be compared to the control petri dish rate of the bacterium. E.g. The percentage of coverage of bacteria on the control dish compared to the other petri dishes with the independent variables on it.

What was investigated?

I selected to investigate the antibacterial properties of different plants around the Jawoyn region. I have done this by boiling the plants and placing the extract on a petri dish with bacteria collected from hands and benches. To measure the outcome, I have chosen to compare the number of bacteria on the agar plate to the control.

Different Plants

Katherine Medicinal Plants

- *Acacia translucens* (p. 101)
- *Centipeda thespidioides* (p. 105)
- *Chenopodium cristatum* (p.106)
- *Cymbonotus lawsonianus* (p. 107)
- *Dianella ensifolua* (p. 108)
- *Eruatamia orientalis* (p. 109)
- *Erythrophleum chlorostachyum* (p. 109)

Jawoyn Medicinal Plants

- *Erythrophleum chlolstachys* (Ironwood) The [redacted] of ironwood is used as medicinal drug.
- *Gyrocarpus americanus* (Shitwood) The [redacted] of Shitwood is used as a medicine.
- *Planchonia careya* (Cocky Apple)

- *Euphorbia australis* (p. 111)
- *Gyrocarpus americanus* (p. 112 & 113)
- *Lavatera plebeia* (p. 114)
- *Mimulas gracilis* (p. 115 & 116)
- *Planchonia careya* (p. 117)
- *Swainsona pterostylis* (p. 120)

The [redacted] of the Cocky Apple are used to prevent bacterial infections.

Preparing the Plants

Ozzie Daylight – "You gotta get da plant, boil 'em up then use the water to wash on yourself and treat sickness"

To prepare you need to crush them up as best you can before you boil the plant in water. Using only the water to wash over body to treat sickness.

Variables

The Independent variable is the solution that is made and spread over the agar plate and the dependent variable is going to be the increase in bacteria. The reasoning for this is due to the fact that the dependent variable changes depending on the independent variable. The Bacteria's growth depends on the solution that is spread across the agar plate.

Independent Variable: Solution placed on agar plate.
Dependent Variable: Bacteria Growth
Uncontrolled Variable: Temperature in cupboard, how tight the dish is sealed.
Controlled Variable:

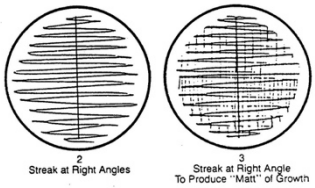
Table 1 – Controlled Variables

Variable	Why it needs to be controlled	How will it be controlled
Amount of water	The same amount of water needs to be controlled because then the concentration of the plant is the same throughout all of the experiment.	50mL
Size of container	The size of the container needs to be kept the same so there is the same amount of surface area for all of the bacteria trials.	Petri Dish
Amount of nutrients	This has to be controlled so that each bacteria has the same living conditions.	The same nutrients and amount will be used

Method

Table 2 - Method

Steps	Justification
1. Collect five different leaf and bark samples and take photos of the different trees for evidence and classification. 2. Separate the samples. Using separate mortar and pestles crush up the plants as much as you can. 3. Place the crushed mixture into 250mL beakers, and using a permanent marker label the beaker with the plant used. 4. Prepare five beakers with the following: o 1g of crushed plant sample o 50mL of water	The plants need to be boiled as this is how Indigenous peoples apply most of the medicinal plants found in the Jawoyn region.

5. Prepare the control beaker with the following: ○ 50mL of water	
6. Setup two Bunsen burners; Heat Mat, Tripod Stand, Gauze Mat, Bunsen Burner, Tongs and PPE. Boil the plant mixtures for five minutes each.	From the boiled mixture Indigenous peoples use the water to cure sickness and prevent bacterial infections.
7. After all of the mixtures have been boiled, get a new 250mL beaker and fill will with 50mL of water.	
8. Dampen a new cotton swab with the water from new beaker and collect bacteria from hands and bench. 9. Then contaminate the petri dish with the cotton swap by swiping side to side all the way down the dish. Turn the petri dish slightly and repeat this four times and after each trial. 10. Repeat steps 8 and 9 for all of the petri dishes.	Bacteria from benches and hands was used as humans interact with it on a daily basis. This means that it has many bacteria that could infect people.  2 Streak at Right Angles 3 Streak at Right Angle To Produce "Matt" of Growth *Figure 2 - How to Contaminate Petri Dish*
11. For each of the plant samples, label two petri dishes with a permanent marker the plant used.	
12. Using a new cotton swab spread the boiled mixture over the agar plates. A different cotton swab needs to be used with a different plant mixture. 13. Repeat step 11 for all of the agar plates.	Using different cotton swab is illuminate cross contamination of the plant samples.
14. Once the samples have been coated in the plant mixture tape the cover to the bottom of the petri dish. 15. Place all of the petri dishes upside down in a cupboard out of human interaction to grow.	
16. Check and analyse the bacteria in five days.	

Plants Used

Figure 3 - Big Leafed Tree

Figure 4 - Shiny Leafed Tree

Figure 3 - Smelly Leafed Tree

Figure 6 - Bauhinia Tree