

## Antibacterial effects of taro (*Colocasia esculenta*) leaf extract on *E. coli*, *S. agalactiae*, and *S. aureus* who thrive on wounds.

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### ABSTRACT

Taro, a staple of the Hawaiian diet, is an integral part of the native culture, dating all the way back to ancient Hawaiian civilization. Plant materials are very beneficial in contributing to alternative medicine today across our world, to help improve the quality of life for humans and increase their life span. Taro has been proven to provide a wide variation of health benefits that help the human body. This study focuses on studying the antibacterial activity of taro leaf extract on three different bacteria: *Staphylococcus aureus*, *Streptococcus agalactiae* and *E. coli*. Dried taro leaves were used in this procedure, grounded to a fine powder, and subjected to a continuous Soxhlet extraction. Based on increasing polarity, a series of solvents (Petroleum ether, Ethyl acetate and Ethanol) were used in three different Soxhlet extraction settings, to isolate organic compounds from taro leaves. Taro extracts were separately concentrated using rotary evaporation and subjected to antibacterial activity. Results from this study showed that the investigated taro leaf extract was successful in inhibiting the growth of *E. coli*, *S. aureus*, and *S. agalactiae*. The petroleum ether extract of taro leaves showed antibacterial activity against *S. aureus* and *E. coli*, but not against *S. agalactiae*, as shown in Figure 3. The inhibition zones obtained from the antibacterial trials were recorded in Table 1. Petroleum ether extract contained something that was active against *E. coli* and *S. aureus*. I am uncertain if there was one compound or the effect of more than one compound that was active. This should be done in a future project, to further investigate this study and the active components of taro leaves. The present research is pertinent in our society and world today, because the development of these products and their active components could help save millions of lives around the world.

Keywords: *Taro*, *Colocasia esculenta*, *soxhlet extraction*, *rotary evaporation*, *antibacterial activity*.

### INTRODUCTION

For a Hawaiian, land and nature is an important part of the culture. The Healthy Ancestor is known as a source of pride, and this is an image that says the natural state of Hawaiians is to be healthy and revitalize their health through their culture (Mcmullin 2005.) Hawaiian heritage and background are a very important part of who I am today. Growing up on the islands of Hawaii, we were taught from a young age that taro is a staple of the Hawaiian diet and is an integral part of the native culture, going all the way back to Hawaiian civilization. In Hawaii, the importance of land is emphasized for a person's spiritual, cultural, and physical health (Mcmullin 2005.) Taro plant holds a special place in Hawaiian culture. "According to Hawaiian legend the first child of the deities was stillborn and was buried. On that very site sprouted taro, which provided sustenance to the subsequent descendants, the Hawaiian people" (Yoshioka et al. 2015.)

Plant materials play a major role in alternative medicine today across our world to help improve the quality of life for humans and increase their life span. The popularity of herbal medicine is increasing continuously over the years, with the rise of newer innovations and improving technology. Many research groups have made significant improvements in the study of plant materials and curing diseases. Traditional medicines are a gift from nature in order to help relieve pain and illness and are still considered as a valuable resource today (Chand et al.

2018.) People of the Pacific are very dependent on herbs to treat many different illnesses (Chand et al. 2018.) Taro, also known as *Colocasia esculenta*, is a root crop that is used as a major starch food in many cultures, particularly among Pacific Islanders (Yoshioka et al. 2015.) The highest percentage contribution of taro to the diet occurs in the Pacific Islands (Gupta and Kumar 2018.) *Colocasia esculenta*, part of the Araceae family, originates from Asia and has been cultivated widely from ancient times in the subtropical and tropical regions of the world (Chakraborty et al. 2015.) Taro has been known for curative properties and utilized for treatment of various medical illnesses such as asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders, and skin disorders (Dutta and Aich 2017.) Toxic in raw form, due to the high content of calcium oxalate in the corms of taro, this plant has been reported to have antimicrobial and antioxidant activity, along with anticancer activity (Chakraborty et al. 2015.)

One of the most important therapeutic discoveries of the 20<sup>th</sup> century is anti-microbial agents, resulting in the development of drug resistance among many hostile pathogenic bacteria (Dutta and Aich 2017.) Several infectious diseases over the years have been known to be treated with herbal remedies, such as taro, throughout the history of humanity (Dutta and Aich 2017.) Taro provides a wide variation of health benefits that help the human body. Such health benefits include boosting the immune system,

reducing cholesterol levels, aiding in weight loss, controlling blood pressure, preventing anemia, rejuvenating the skin, and acting as an antioxidant to prevent cancer. Taro leaves are also a rich source of protein, ascorbic acid, dietary fiber, and other important minerals including, thiamin, riboflavin, iron, phosphorus, zinc, vitamin B6, vitamin C, niacin, potassium, copper, and manganese (Shekade et al. 2019.) Taro leaves are exceptionally high in folate, making nutritious especially for pregnant and lactating women (Gupta and Kumar 2018.) High potassium content of taro leaves makes it a good coagulant to prevent excessive bleeding or hemorrhages (Gupta and Kumar 2018.) Taro has a long history of usage in traditional medicine in several countries across the world, especially in the tropical and subtropical regions (Al-Kaf et al. 2019.) Taro is also strongly recommended for general medicinal purposes and specifically for treating wounds and burns, due to their rich antioxidant content (Al-Kaf et al. 2019.)

Research on taro leaves report that the ethanolic extract of taro might become a useful agent in the treatment of bacterial and fungal diseases (Dutta and Aich 2017.) Some studies have also suggested that taro leaves have analgesic and anti-inflammatory properties (Shekade et al. 2019.) Taro leaves, dried up, can be used as a functional powder which has a potential to improve overall health in humans (Shekade et al. 2019.) However, further studies are necessary to prove these abilities. In farming, taro leaves are also known to provide a protein-rich fodder, which allows farmers to reduce the use of expensive soybean meal (Hang et al. 2018.) Soybean meal is the most widely used protein source in pig production in tropical countries (Hang et al. 2018.) Promising and encouraging results from previous studies on taro leaves tells researchers the importance of purifying and isolating the active components for further clinical approaches in the future (Chakraborty et al. 2015.)

This study focuses on studying the antibacterial activity of taro leaf extract on three different bacteria: *S. aureus*, *S. agalactiae* and *E. coli*. Once extracts are recognized to have antibacterial properties, they will be isolated and identified.

## MATERIALS AND METHODS

### i. Taro leaf powder

The study performed is a modification of a study found in literature (Dutta and Aich 2017.) Dry Kapuso Brand Taro Leaves were purchased through the Etsy website and were grounded to a fine powder using a Magic Bullet Blender. The powder was stored at room temperature in the lab until further use.

### ii. Pilot Project - Soxhlet Extraction

First, the study was done using a Pilot Project and then was expanded to a Major Study. The pilot study

extraction was carried out by using a Soxhlet apparatus, which provided an inexpensive method for recovery of phytochemical and had been proven to provide high extraction (Keshav et al. 2019.) Two Soxhlet apparatuses were used during this experiment for better time efficiency, as shown in Figure 1. Approximately ~50 grams of taro powder was weighed and placed into a thimble. The thimble during Soxhlet extraction ensures that the rapidness of the solvent does not allow for any solid material to enter the round bottom flask. 250mL of the solvent Ethanol was measured using a graduated cylinder and poured into a 500mL round bottom flask. A stir bar was added to the round bottom flask. The round bottom flask was then placed inside of a heating mantle, the heat source that sits on top of a hot plate, used only for stirring purposes. Once the solvent was inside of the round bottom flask, the Soxhlet apparatus was attached. The thimble was then carefully placed inside of the Soxhlet apparatus and the condenser was connected. This process was repeated for both Soxhlet apparatuses. Using one hose, water enters through the bottom of the condenser and water comes out using another hose through the top of the condenser, connecting the two condensers in the middle. In order to save water during the experiment, since each trial took 16 hours to complete, a bucket of water was filled up with a garden pump placed inside to recycle the water coming out of the second condenser back through the cycle of the first condenser. Ice was placed inside of the water bucket periodically, to ensure that the water was staying cold enough. Each piece of the apparatus was greased before connecting with a clamp and extra ring stands were used for sturdy support. Once everything was set up, the heating mantle and water were turned on, and the stir bar was activated to begin the reflux process.

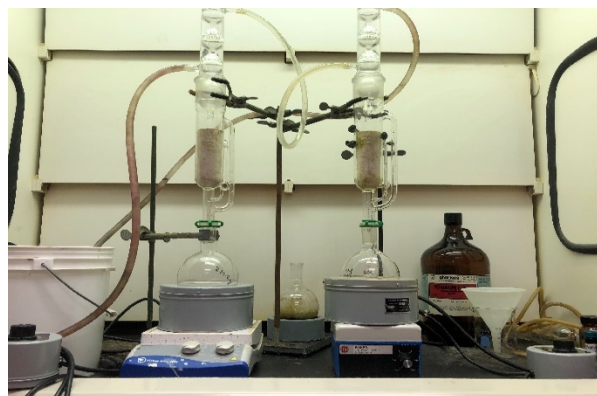


Figure 1: Soxhlet extraction apparatus

### Rotary evaporation

Extract was collected in round bottom flasks and then concentrated in vacuum using a rotary flash

evaporator (Dutta and Aich 2017.) During this process, the solvent was separated from the extract and collected inside of another round bottom flask as shown in Figure 2. Approximately 1.217 grams of the dried Ethanol extracts were dissolved in 3.0 mL of dimethyl sulphoxide (DMSO) and subjected to antibacterial activity.



**Figure 2.** Rotary evaporator

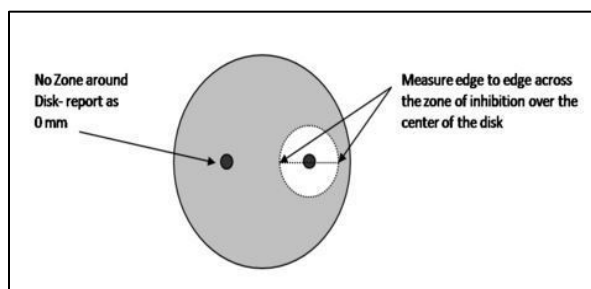
### Antibacterial Study

Testing the discs for antibacterial activity was done by performing the modified agar disc diffusion method (Dutta and Aich 2017.) In order to reactivate the bacteria *S. aureus* and *S. agalactiae*, first the metal band on the vial was removed, along with the gray butyl stopper. Using a micropipette, 1.0mL of rehydration medium was removed from the test tube and added to the powdered culture in the vial. This solution was mixed gently using a sterile pipet, then the rehydrated culture was removed from the vial and transferred back into the tube containing the remaining rehydration medium. Cultures were incubated overnight at 37°C, before initiating cultures using the inoculation technique. An inoculating wire loop was used to take a loopful of isolated colony of the overnight grown culture and placed inside of test tubes filled with broth premade already, courtesy of Dr. Frye. The inoculating wire loops were sterilized over the Bunsen burner before and after every use.

To prepare the Mueller Hinton Agar media, approximately 38 grams of media was placed inside a 2L flask. The flask was filled with 1,000mL of Deionized water. A stir bar was added inside of the flask, and aluminum foil was placed on top of the flask. The flask was then placed onto a hot plate, with the heat to 400°C and the stir bar to 300RPM. As soon as the media began to bubble about halfway up the flask, a hot pad was used to remove the flask from the hot plate. The stir bar was removed from the flask, autoclave tape was added around the foil and the flask was then autoclaved. From the autoclave, the flask of media was placed into an incubator set at 45°C. Once the media was cooled down, the agar was then poured

onto twelve plates.

The bacterial study was conducted as follows, once the media was solidified, each set of plates were infected with a different microorganism. The microorganisms used in this experiment were *S. aureus*, *S. agalactiae*, and *E. coli*. Paper discs were impregnated with 15 microliters of ethanolic extract dissolved in 3.0 mL of DMSO and placed individually on each plate. Three impregnated discs were placed on each plate, far enough from each to try to avoid overlapping from rings of inhibition. DMSO was used as the control during this experiment. The petri dishes were then incubated for 24 hours at 37±2°C, in order for the bacteria to grow. After 24 hours, the plates were taken out of the incubator and checked to see if any zone of inhibitions were produced. The zone of inhibition was measured by using a ruler and measuring from edge to edge, over the center of the disc as shown in Figure 3. Each test was completed three times to ensure reliability and validity.



**Figure 3.** Measuring zones of inhibition (Hudzicki 2009.)

### iii. Major Study

Based on the results of the Pilot Study it was decided to do the Soxhlet extraction, using a series of solvents rather than with just one solvent. It was decided to use Petroleum ether, Ethyl acetate and Ethanol in three different Soxhlet extraction settings in the order of increasing polarity of the solvents. The reasoning behind the solvent series was to isolate organic compounds from taro leaves, separated based on the polarity of each solvent.

#### Soxhlet Extraction

Using the same method as shown above in the Pilot Project, ~100 grams of finely grounded taro powder was extracted in 500 mL of Petroleum ether. The extract was separated and the same remaining powder was then extracted into 500 mL of ethyl acetate using the Soxhlet apparatus. Lastly, a third extract of the remaining powder was completed using 500 mL of Ethanol.

### Rotary Evaporation

All three extracts were separately concentrated using rotary evaporation, as shown in the Pilot Project above. The dried extracts were then dissolved in

DMSO. Approximately 1.264 grams of the Petroleum ether extract were dissolved in 3.0 mL of DMSO, 1.300 grams of the Ethyl acetate extract were dissolved in 3.0 mL of DMSO and 1.271 grams of the Ethanol extract were dissolved in DMSO.

#### Antibacterial Study

The bacterial study was completed by using the same method explained above in the Pilot Project. However, this time the paper discs were impregnated using 60 microliters of each extract placed individually on each plate. The reason for increasing the amount of extract placed on each disc this time was to ensure that the discs were completely soaked in extract.

## RESULTS

### i. Pilot Project

In our study, the results showed that the investigated taro leaf extract was successful in inhibiting the growth of *E. coli*, *S. aureus*, and *S. agalactiae*. The ethanolic extracts of the taro leaves, did not show any antibacterial activity against *S. aureus*, *S. agalactiae* and *E. coli*.

### ii. Major Project

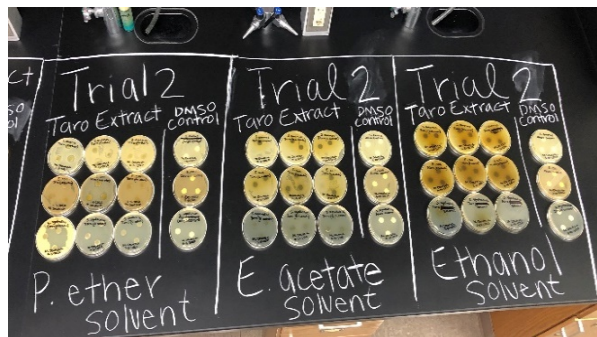
Similar to the Pilot Project, our results showed that the investigated taro leaf extract was successful in inhibiting the growth of *E. coli*, *S. aureus*, and *S. agalactiae* in this study. The Petroleum ether extract of taro leaves showed antibacterial activity against *S. aureus* and *E. coli*, but not against *S. agalactiae*, as shown in Figure 4. The inhibition zones obtained from the antibacterial trials were recorded in Table 1.

**Table 1:** The zone of inhibition (mm), shown by three different bacterial species with Petroleum ether, Ethyl acetate and Ethanol extracts.

Extract	Bacterial Species		
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. agalactiae</i>
Control	No resistance	No resistance	No resistance
Ethanol	Bacteria is resistant	Bacteria is resistant	Bacteria is resistant
Ethyl acetate	Bacteria is resistant	Bacteria is resistant	Bacteria is resistant
Petroleum ether	2.2 mm = AVG zone of inhibition	2.8 mm = AVG zone of inhibition	Bacteria is resistant

## DISCUSSION

After the Pilot Project, I realized that the ethanolic extract did not show any bacterial activity. This extract may have contained more than one molecule, but I am not sure if there was an active molecule. The extracts may have also been hindered by the presence of



**Figure 4:** The petri dishes from the major project showing the bacterial growth in the presence of different extracts: Petroleum ether, Ethyl acetate, and Ethanol.

larger amounts of molecules that are not active by the molecules I was studying. Extract active compounds into three different solvents with different polarities. First, extracted into petroleum ether, as Table 1 suggests, extract contained something that was active against *E. coli* and *S. aureus*. When increasing the polarity of the solvent to Ethyl acetate, no compounds were active.

The next step would be taking a sample of the Petroleum ether extract and separating it into individual components using a chromatography technique, identifying the molecule or molecules, which are active against *E. coli*, and *S. aureus*. However, due to the time and facility restrictions due to the COVID-19 pandemic, I was not able to perform this part of the study. With the current results, I am unsure if there was one compound or the effect of more than one compound that was active. This should be done in a future project, to further investigate this study and the active components of taro leaves.

Even though the Petroleum ether extract was active against these two bacteria, when looking at the zone of inhibitions this suggests that it is necessary to take a better measurement of the antibacterial activity of the abstract. As the results suggest, the ethanolic and Ethyl acetate taro leaf extract evaluated in this study did not exhibit antibacterial activity against *S. aureus*, *S. agalactiae*, and *E. coli*. The Petroleum ether extract of taro exhibited antibacterial activity against *S. aureus* and *E. coli*, but not against *S. agalactiae*. Due to the results obtained in the study, we can assume that the ethanolic leaf extract of taro, might become a useful component in the treatment of bacterial diseases (Dutta and Aich 2017.)

Taro leaves contain flavonoids which are a type of phenolic compound that are commonly found in plants. Certain flavonoids found in taro leaves include; orientin, isoorientin, isovitexin, vicenin-2, vitexin and luteolin (Bakr et al. 2017.) Flavonoids are responsible for helping the body function more efficiently and help protect the body, as antioxidant agents. Traditional

medicines are not only just a gift from nature, but they are the future of saving lives and doing so, while using less harmful chemicals and releasing these into our environment and atmosphere. Further well-designed studies are necessary to throw light on the various uses of herbal drugs for the benefit of mankind (Dutta and Aich 2017.)

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