INVESTIGATING THE EFFECTS OF NATIVE SONORAN DESERT PLANTS ON ENHANCING THE EFFECTS OF ANTIBIOTICS AGAINST *E. COLI*

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ABSTRACT

Antibiotic resistance is one of the most serious public health issues that the globe is facing today. Every year, around 2.5 million people in the United States contract an antibiotic-resistant infection, with 35,000 people end up dying. To address this problem, this study investigated the pharmaceutical potential of 4 different Sonoran Desert plants and determined whether they could enhance the effects of the antibiotics (Erythromycin, Ampicillin, Penicillin).

This project was conducted using the following methods: First, to obtain the ethanoic extracts of *Larrea tridentata, Fouquieria splendens, Cercidium microphyllum, and Prosopis glandulosa* leaves, rotary evaporation was performed. Secondly, bacterial dilutions were prepared following the 0.5 Mcfarland standard. Thirdly, paper discs were prepared and grouped into 2 treatments. Treatment 1 received 10µL of the plant extracts only while treatment 2 received 10µL of antibiotics and 10µL plant extract. Discs receiving antibiotics only were used as a control. Lastly, Kirby-Bauer Sensitivity Assay was performed, and zones of inhibition were measured using a digital caliper.

The results of the experiment showed that *E. coli* was susceptible to all plant extracts with recorded average zones of inhibition higher than 16mm. Antibiotics mixed with plant extracts showed a significant increase in its effects as shown by zones of inhibition higher than 16mm. This was also supported by the t-test results for all treatments with p value of <0.05. However, the effects of *Larrea tridentata* and *Fouquieria splendens* were diminished when mixed with Penicillin. This research presents those Native plants from the Sonoran Desert have antimicrobial properties.

RATIONALE

Are antibiotics still effective? Antibiotics have played a major role in the survival of the human race. Fighting and eliminating pathogenic bacteria has been a major concern in the field of health and medicine. Although humans were able to combat these pathogens, bacteria evolved to be resistant to antibiotics. The promotion of bacteria on converting less resilient is still an unknown method, regardless of modern technology. Nevertheless, this project investigated a way to lessen antibiotic resistance in bacteria using several native Sonoran Desert plants.

Antibiotic Resistance

According to the Centers for Disease Control and Prevention (CDC), antibiotic resistance occurs when bacteria develop defenses to respond to antibiotics. In what is sometimes called an evolutionary arms race, bacteria develop ways to stop the drugs that are designed to kill them. When bacteria cease on having a reaction to antibiotics, bacteria can continue to grow inside the human body and once a large enough number of the bacteria are present, the cells release toxins. Those toxins damage your body's tissue and consequently make you feel ill. The CDC considers the growing threat of antibiotic resistance to be one of the world's most urgent public health problems. It can affect people at any age, and threatens our healthcare, veterinary, and agricultural industries. Antibiotics are the remedy to cure patients with bacterial infections, if their effectiveness is lost, the chances to cure individuals are also lost.

Moreover, bacteria can become antibiotic resistant because of a process known as natural selection. Natural selection is the process where organisms better suited to their environment will survive and consequently reproduce. When bacteria are often and repeatedly exposed to an antibiotic, the population will eventually evolve to survive through genetic mutation and selection. To be more specific, the genes in bacteria cells will randomly mutate and some mutations may help the cells survive in the face of antibiotics and those individuals will keep reproducing. As a result, the frequency of antibiotic resistance genes increases in the population. Because of this process, when these pathogenic bacteria are again exposed to the same antibiotic the population of cells are capable of resisting the effects of the antibiotic. Mutations protect the bacterium cell, while the bacteria without the mutation will either die or not be capable of reproducing anymore. After suiting to survive antibiotics, bacteria can continue to reproduce while it passes their acquired genetic mutations to their offspring.

There are two existing ways that bacteria become antibiotic resistant. One is through mutations that occur in the DNA (deoxyribonucleic acid) of the cell during replication. While the other way is, when bacteria acquire resistance through horizontal gene transfer.

Antibiotic resistance has become one of the biggest public health challenges. Every year in the U.S 2.8 million people get an antibiotic-resistant infection while more than 35,000 people die from antibiotic resistant infections. Despite how advanced our modern technology is, there is still no known method or procedure for how to promote bacteria to become less resistant. A potential method to combat antibiotic resistance lies in the use of plants and their antimicrobial properties. Five plants of interest are described below.

Mesquite Plant

The mesquite plant (*Prosopis glandulosa*) is native to the Southwestern United States and Mexico. Mesquite leaves are used for producing tea that aids in headaches and stomach trouble. In addition, the Arizona Daily Independent News Network reported *Prosopis glandulosa* to have various medical potentials such as being effective at curing sore lips, chapped skin and treating venereal disease. Also, some use the leaflets of the tree to make a sunburn lotion.

Creosote Bush

Larrea tridentata commonly known as creosote bush is a native Sonoran Desert plant located in the Southwestern United States and Mexico. Creosote can live for up to 100-200 years and still own their authentic odor. The plant's medical properties were first discovered by indigenous thousands of years ago because of creosote's strong smell. Since then, the waxy leaves and stems of the bush have been used to make teas and other remedies to cure at least 14 afflictions and diseases, (Schalau, 2005).

Creosote is not only an antimicrobial bush but it is also rich in vitamins, minerals, digestive enzymes, antioxidants, and other Phyto-nutrients. Despite that it may cause liver and kidney damage when concentration is too high, many still use creosote to cure illnesses such as fevers, colds, stomach pains, diuretic, arthritis, sinusitis, and anemia (Ethno Herbalist, 2020). Also, creosote is a natural general pain killer and an anti-diarrheal. Moreover, besides its natural beauty, what also makes this plant so magnificent is that it can treat cuts and bacterial or fungi infections.

Creosote is rich in simple biosphere lignans and tricyclic lignans, which result in beneficial plant pharmaceutical potentials. These compounds are powerful agents against human immunodeficiency viruses, human papillomavirus, cancer, neurodegenerative diseases, and symptoms of aging (Journal of Traditional and Complementary Medicine, 2015).

Palo Verde

There are many types of palo verde trees throughout the US and Mexico, but *Cercidium microphyllum* in specific is one of the most prominent trees in the Phoenix area. This tree was named after one of its most notable characteristics, its color. According to Arizona State University College of Pharmacy, palo verde is safe and nontoxic for both people and animals. Regarding the usage of this tree, there is not much information on its medicinal use. Meanwhile, more research ought to be done on this plant since there is no record of any significance that the plant may have on the medical field or if it was ever used to treat any illness.

Ocotillo

Fouquieria splendens, known as ocotillo, is a warm desert plant native to the southwestern United States and Northwest Mexico. This plant is quite common all throughout Arizona deserts, ocotillo can not only be found in the desert but also in the gardens of many. The flowers and leaves

of ocotillo have been used to treat spasmodic coughs and as a stimulant. Despite its bitter taste, ocotillo is a decongestant, stimulant, cholagogue, expectorant, and mild emmenagogue. Due to the fact that Ocotillo is a lymphatic decongestant, it makes the bush helpful in seasonal colds, spasmodic coughs, and low immunity. There is no doubt that this plant is truly fascinating; the best of all is that if they live under adequate conditions, they can live for up to 60 years! Furthermore, one more interesting thing about Ocotillo is that it can increase the uptake of dietary fats from the portal vein into the lymphatic tissue of the pelvis. As a result, it improves digestion, assimilation, use of fats, and relieves stagnancy of blood in the area (Blogroll,2014).

This research will investigate which among the four native Sonoran Desert plants will have the greatest effect on diminishing the antibiotic resistance of *E. coli.*

STATEMENT OF THE PROBLEM

This project will investigate the effects of several native Sonoran Desert plants, *Prosopis glandulosa, Larrea tridentata, Cercidium microphyllum, Fouquieria splendens* on diminishing the antibiotic resistance of *E. coli.*

Specifically, this project addressed the following questions:

1. Which among the four native Sonoran Desert plants will have the greatest effects against *E. coli?*
2. Which among the four native Sonoran Desert plants will enhance the effects of the different antibiotics?

HYPOTHESIS

This project has the following hypothesis:

If *E. coli* is exposed to different extracts derived from native Sonoran Desert plants, antibiotic resistance should diminish as most of the plants selected have antimicrobial properties, such as *Prosopis glandulosa, Larrea tridentata,* and *Fouquieria splendens.*

VARIABLES

Independent: Leave extract from *Prosopis glandulosa, Larrea tridentata, Cercidium microphyllum,* and *Fouquieria splendens*

Dependent: Zone of Inhibition (antibiotic resistance)

Constant: Amount of extract, concentrations of the antibiotics, temperature (37^{o} C), type of bacteria

METHODS

Materials and Procedures

Selection and plant collection. Samples of *Prosopis glandulosa, Larrea tridentata, Cercidium microphyllum, and Fouquieria splendens* were collected from the Sonoran Desert in surrounding Yuma, Az. **Drying and grounding of plants.** Plants will be allowed to dry or dehydrate for 5 days. After dehydrating the plant samples will be grounded using a coffee grinder.

Extraction using (95%) ethanol. Plants leaves will be soaked in a jar with 20% distilled water and 80% ethanol solution for 7 days.

Collection of phytochemical constituents. The solvent will be removed from the extract using a rotary evaporator. The extract will be stored at room temperature until use.

Bacteria Samples. A test tube culture of Biosafety Level K-12 of *E. coli* will be the test subjects in this experiment. The bacteria will be purchased from Carolina Biological. To obtain more pure colonies of the samples, the streak plate method will be performed.

Preparation of bacterial lawn. Bacterial dilution of *E. coli* will be done based on the McFarland Standard. *E. coli* will be evenly spread across the Mueller-Hinton agar plates using a sterile bacterial spreader.

Preparation of antibiotic discs. This process will involve two groups: Sterile paper discs with plant extract only and sterile paper discs with antibiotic + plant extracts. Sterile antibiotic discs will be soaked in Penicillin, Ampicillin, Erythromycin and Neomycin with a concentration of 10 mg/ml. 10 µL of each plant extract will be added to the antibiotic discs. Antibiotics, sterile water, and ethanol will be used as controls.

Kirby-Bauer antibiotic sensitivity assay. Kirby-Bauer antibiotic sensitivity assay will be used to observe and record the zones of inhibition. Inoculated Mueller-Hinton plates with prepared discs will be stored in a digital incubator overnight at 35^{o} C. The diameter of the zones of inhibition will be measured using a digital caliper.

Minimum inhibitory concentrations and OD using spectrophotometer. Minimum inhibitory concentrations will be measured using a microplate and spectrophotometer under optical density of 600 nm.

Potential Risks and Safety Precautions

Potentially hazardous substance. The live bacterial sample tube of *E. coli* will be ordered from Carolina Biological with a Biosafety Level 1l.

Site of experimentation and biological containment. The experiment will be conducted within the science classroom with all persons following safety guidelines. The laboratory sink and countertop will be utilized in performing the experiment. Petri dishes will be kept closed throughout the experimentation to avoid contamination. A digital incubator will be used to control the temperature for the growth of the bacteria.

Procedures to minimize risk. The researcher will be using PPE (gloves, mask, goggles, and laboratory coat) in performing the Kirby-Bauer Antibiotic sensitivity assay. Petri dishes will be kept sealed at all times. The laboratory sinks and the counter top will be sanitized before and after the experimentation.

Statistical Tools

Paired t-test. Data collected from the Kirby-Bauer antibiotic sensitivity assay was analyzed using paired t-test test to see if there is a significant difference among the tested treatments.

Percent increase. This will be done comparing the zones of inhibition between the treatment receiving plant extract + antibiotics and antibiotics only. The formula below will be utilized:

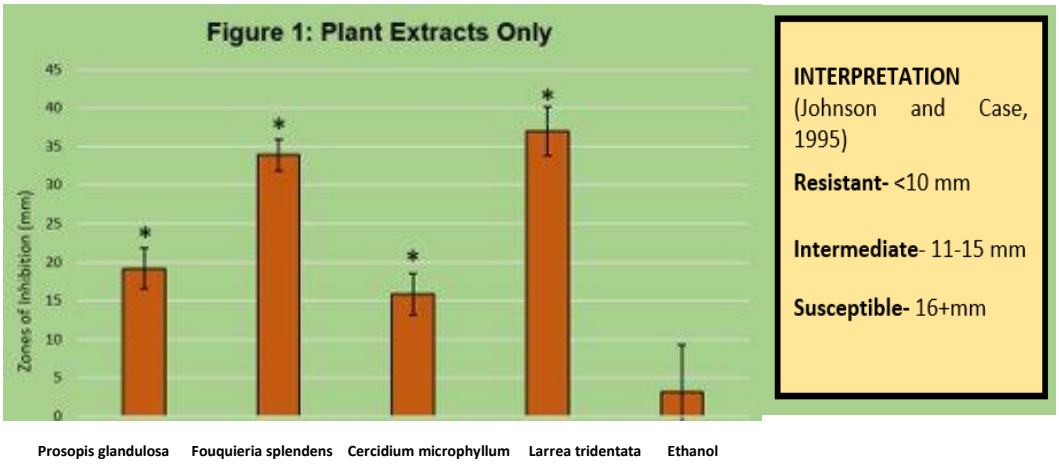
$$\%\text{increase} = \frac{Z_1 - Z_2}{Z_2} \times 100$$

Where: Z_1= Zone of inhibition with plant extract + antibiotics

Z_2= Zone of inhibition with antibiotics only

RESULTS

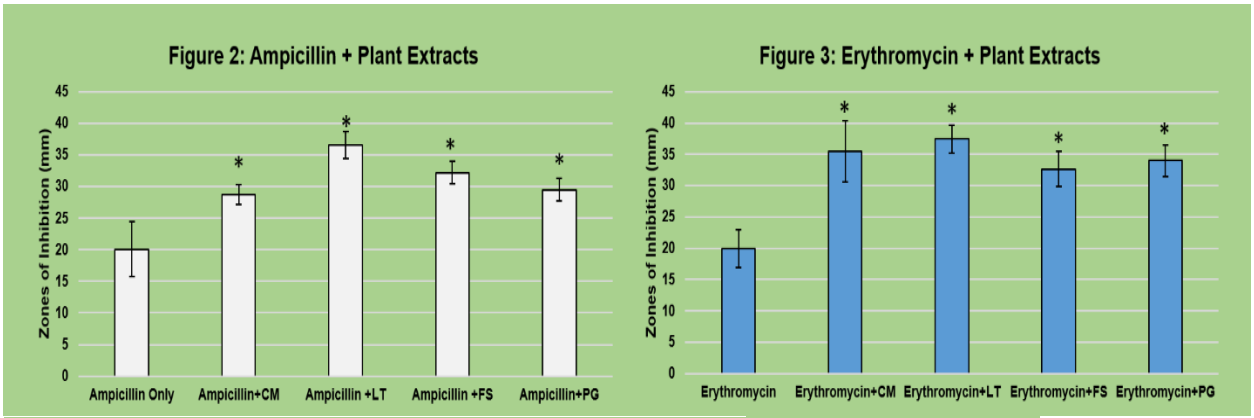
Results Part 1: Plant Extracts Only

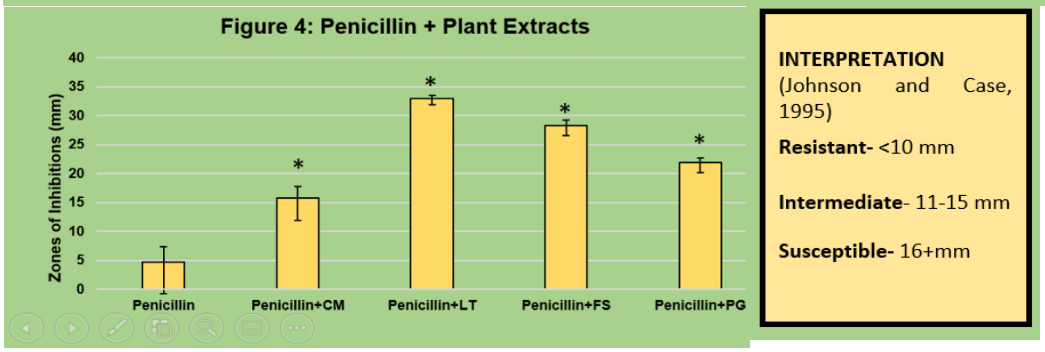


** Denotes significant difference between the plant extracts and ethanol with $p<0.05$ (paired t-test)*

- All four plant extracts were able to kill *E. coli* with average zones of inhibition higher than 16mm.
- *Larrea tridentata* (Creosote) exhibited the highest antibacterial property with an average zone of inhibition of 37mm.
- Results are validated by paired t-test with p-value of less than 0.05.

Results Part 2: Plant Extracts+ Antibiotics





** denotes significant difference between the antibiotics and antibiotics with plant extracts with $p<0.05$ (paired t-test)*

Results Part 3: Percent Increase

Plant Extracts + Ampicillin

Point of Reference: Ampicillin only 20.1 mm

Treatments	Ampicillin + CM	Ampicillin +LT	Ampicillin +FS	Ampicillin +PG
Average Zones of Inhibition	28.775	36.55	32.175	29.5
Percent Increase	43.16%	81.84%	60.07%	46.77%

Plant Extracts + Erythromycin

Point of Reference: Erythromycin only 19.925 mm

Treatments	Erythromycin + CM	Erythromycin + LT	Erythromycin + FS	Erythromycin + PG
Average Zones of Inhibition	35.475	37.45	32.65	33.975
Percent Increase	78.04%	87.95%	63.86%	70.51%

Plant Extracts + Penicillin

Point of Reference: Erythromycin only 4.7 mm

Treatments	Penicillin + CM	Penicillin + LT	Penicillin + FS	Penicillin + PG
Average Zones of Inhibition	15.775	33.025	28.35	21.875
Percent Increase	70.20602219	85.76835731	83.42152	78.51429

The results of the experiment showed that *E. coli* was susceptible to all plant extracts with zones of inhibition being greater than 16mm. On the other hand, for treatment two the results showed that Erythromycin and Ampicillin were the only antibiotics to be efficient on diminishing antibiotic resistance of *E. coli.* Finally, for treatment three the outcomes revealed that the individual activity of all antibiotics were highly improved with the presence of plant extracts. As compared with the control treatments, all native Sonoran Desert plants showed promising results on enhancing different antibiotics as well as lowering antibiotic resistance of *E. coli*.

DISCUSSION

Answer to Problem 1: Based on the results of the experiment, among the four native Sonoran Desert plants that were tested (*Prosopis glandulosa, Fouquieria splendens, Cercidium*

microphyllum, Larrea Tridentata), *Fouquieria splendens* and *Larrea tridentata* were the most effective against *E. coli.*

Answer to Problem 2: The results showed that among the four native Sonoran Desert plants that were tested, all enhanced the effects of different antibiotics (Ampicillin, Erythromycin, Penicillin).

CONCLUSION

In conclusion, based on the results and data analysis, my hypothesis was proven to be correct. Native Sonoran Desert plants were able to enhance the effects of antibiotics against *E. coli.* Meaning that the tested Native Sonoran Desert plants possess antimicrobial properties capable of increasing the effectiveness of certain antibiotics.

Observations After Experiment:

Ampicillin and Erythromycin, each from a different class of antibiotics, showed promising results on lowering antibiotic resistance of *E. coli*. This validates previous studies that these antibiotics are effective against *E. coli*. On the other hand, certain plant extracts' effects were slightly diminished by the presence of Penicillin. When *Larrea Tridentata* and *Fouquieria splendens* were combined with Penicillin (the antibiotic that showed resistance) the results were lower than their individual activities. *Larrea tridentata* exhibited the highest antibacterial property with an average zone of inhibition of 37mm. However, when it was exposed to Penicillin the average zone of inhibition lowered from 37mm to 33mm. Similarly, *Fouquieria splendens* exhibited an average zone of inhibition of 33mm. Nevertheless, when Penicillin was added the average zone of inhibition lowered to 28mm.

THEORY

E. coli is a gram-negative bacteria, its outer membrane prevents it from absorbing penicillin, leading to resistance. A possible explanation to why the individual activity of plant extracts resulted to be most efficient than when combined with Penicillin, is because penicillin properties destroyed some active components found within the extracts. Since Penicillin is an acid, it's possible that *Larrea tridentata* and *Fouquieria splendens* are acid labile, and the acids lower their pH. The potency of plant extracts would be diminished as a result.

Credits: *This theory was developed with the help of Dr. Molnar, Professor, School of Natural Resources and the Environment Associate Director, Southwest Center for Natural Products Research at the University of Arizona.*

FUTURE PLANS AND POINTS FOR IMPROVEMENT:

1. Research other native Sonoran Desert plants and test their antibiotic properties.
2. Identify the phytochemical constituents (saponins, tannins, glycosides, flavonoids, etc.) of the tested herbs that contribute to their antibiotic properties.
3. Perform more Kirby-Bauer Antibiotic Sensitivity Assay Test and Synergy Testing to assess the *in vitro* interaction of antimicrobial combinations.
4. Perform an MIC Test (Minimum Inhibitory Concentration).
5. Test more antibiotics as well as bacteria to learn more about their interactions with different plant extracts.

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