

Are Plant-Based Phytochemicals an Alternative to Antibiotics in Combating Drug Resistance in
Bacteria?

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Category of Research: Phytochemicals and Inhibition Effect

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1. Introduction

1.1. Abstract

More than 4.8 million U.S. residents suffer from food poisoning every year, and some cases lead to adverse effects such as unusual stools, diarrhea, stomach aches, vomiting, and, in some cases, paralysis and death. The solution used, antibiotics, is not optimized for the homeostasis of the human body. They kill off beneficial bacteria and leave the body worse off. Ayurvedic, Jamu, and other eastern traditional medicines showcase a plethora of Plant Derived Substances(PDS) that each have benefits with little to no side-effects. The hypothesis is *Hibiscus Sabdariffa* and *Azadirachta indica* (*Neem*) can be used as an alternative to antibiotics and the purpose of the study is to evaluate the antibacterial activity of the ethanolic extract of *Hibiscus Sabdariffa* and *Azadirachta indica* The average *Azadirachta indica* and *Hibiscus Sabdariffa* inhibition was on par with Tetracycline. According to the present study, *Hibiscus Sabdariffa* and *Azadirachta indica* can be used as an efficacious alternative to drug resistance in bacteria.

Keywords: antibiotics, enterobacteria, Plant-Derived Substances, *Hibiscus Sabdariffa*, *Azadirachta indica*, drug resistance

1.2. **Background**

1.2.1. **Background**

In the 21st century, 600 million infections and 420 000 deaths are caused by foodborne pathogens worldwide each year, says the World Health Organization (2015)(WHO¹). Antibiotics, the most effective drugs against microbial infections in the 1950s, are recently losing their efficacies as most microorganisms have acquired resistance. Despite the negative side effects of antibiotics on human organs, the intensive use of antibiotics has led to the emergence of what is called multidrug resistant (MDR) bacteria which are now rising remarkably all over the world and become an international public health threat, as stated by E.M. Abdallah (2016). Moreover, such pathogens have negative impacts on the economy worldwide. The 2019 World Bank statement stated that an estimated 95.2 billion per year, and the annual cost of treating foodborne illnesses is estimated at 15 billion, stated by the World Health Organization(2009)(WHO²).

1.2.2. **Antibiotics Background**

Currently, a disturbing consequence of antibiotic treatment is the long-term persistence of antibiotic resistance genes, for example, in the human gut. C. Jernberg et al. (2010), state how antibiotics are becoming less efficacious over time. This proposes a daunting problem, as antibiotics that helped us turn a 2.5% mortality rate to a 0.0005% mortality rate of the common cold, are now becoming ineffective against it (CDC). Antibiotics hurt and compromise the microbiome, causing diarrhea, inflammatory bowel disease, obesity, cancer, and others. The prolonged exposure to antibiotics causes many if not all the aforementioned issues. The plant alternatives are much better since they are natural, healthy, and not synthetic, while antibiotics are not (Jernberg, 2010). Increasing antimicrobial resistance is a growing threat to human health and is mainly a consequence of excessive use of antimicrobial agents in clinical medicine.

1.2.3. Hibiscus Sabdariffa

Hibiscus Sabdariffa is a well known multipurpose medicinal plant, which belongs to the family Malvaceae. It is an annual tropical short shrub and widely prevalent in many tropical and subtropical regions in the world. Due to the presence of many phytochemical compounds, hibiscus sabdariffa has been found to have antioxidant, hypotensive, hypocholesterolemic, immune-modulated, hepatoprotective, renoprotective, diuretic, anti-obesity, antiurolithic, antidiabetic, antimicrobial and anti-cancer properties without any significant genotoxic effects. Calyces of roselle is a famous public beverage in Sudan employed traditionally for the treatment of many ailments, such as respiratory tract infections, colds, fevers, hypertension and malaria.

(Abdallah).

1.2.4. Azadirachta Indica (Neem)

V.S. Kumar (2013) explains how Neem is a member of the mahogany family, Meliaceae. It is today known by the botanic name *Azadirachta indica A. Juss.* According to Yarmohammadi (2021), the Neem plant is widely used in Indian medicine across many applications, including body/face/hair care, medicines, food and oral products. Neem is used for its antioxidant and antibiotic effects, anti-malarial antimalarial, slight antifungal, antiviral, anti-hypertension, anti-hyperlipidemia, anti-obesity, anti-cancer, antidiabetic effects and promotes liver, brain, immune system, and nervous system health. Many use the oil as a repellent against pests and mosquitos, especially those carrying diseases and malaria.

PDSs (Hibiscus/Neem)

Phytochemicals	Effects	Uses
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<ul style="list-style-type: none"> ● Polyphenols(Anthocyanins, Polysaccharides, Organic Acids) ● Tannins ● Alkaloids ● Limonoids ● Flavonoids ● Phenols ● Catechins ● Nimbins <p>Sandhir et al(2021)(Sandhir), Riaz et al(2018)(Riaz)</p>	<ul style="list-style-type: none"> ● No disruption in cell membrane ● Inactivates efflux pump ● Inhibits quorum sensing ● Inactivation of enzyme modification ● Prevention of drug alteration ● Prevention target bypass ● Prevention of biofilm formation <p>(Al Sheik)</p>	<ul style="list-style-type: none"> ● Biological QC ● Biological Characterization ● Assessing Bioavailability ● Bioactivity Guided Fractionation ● Elucidating Bioavailability <p>(Al Sheik)</p>
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1.3. Purpose

The purpose of this experiment was to test the antibacterial effect of *Hibiscus Sabdariffa* and Neem.

1.4. Hypothesis

The hypothesis is if the enterobacteria is inoculated with Hibiscus and Neem, then the bacteria will be killed.

1.5. Impact

The impact of my research is that it provides an alternative to antibiotics.

2. Experimental Design

2.1. Description of Experiment

The goal of the experiment was to determine the antibacterial properties of two plants, *Hibiscus Sabdariffa/Sinensis* (Hibiscus) and *Azadirachta Indica* (Neem).

2.2. Materials List

- *Sporosarcina Ureae*, Agar, Slant(1)
- Nutrient Agar, Prepared Media Plates, 100 x 15 mm, Pack of 10 (2 packs)
- *Enterobacter aerogenes*, Living, Nutrient Agar, Slant (1)
- Gram Stain Comparison Set, Living(1)
- Economy Antibiotic Disk, Tetracycline, 30 mcg(1)
- Ethanol (25 mL)
- Hibiscus Powder (1g)
- Neem Powder (1g)
- Loop Swab (1)
- Box of Sterile Gloves (1)
- Tape
- Black Sharpie
- Red Sharpie
- Green Sharpie
- Lighter (For sterilization)
- Metal Tweezers
- Paper Tabs (100)

2.3. Procedure

2.3.1. Preparation of Disc for Antibacterial Activities

Kirby Bauer Disc Diffusion Method

This method involves the diffusion of antimicrobial agents from a paper disc into an agar medium that has been inoculated with the test microorganism.

In this case, the sterile blotting paper disc (10 mm) was soaked in the diluted ethanol extracts in such a concentration that the amount of solution absorbed by each disc was 5 mg of each extract of herbal preparations (Hibiscus Sinensis and Neem).

2.3.2. Step-by-Step Procedure

Preparation of plates

- With sterilized gloves, Measure one gram of both Hibiscus powder and Neem powder, and extract with 5 mL of ethanol each.
- Set aside, and draw 4 quadrants on the top of the Petri Dish, labeling with red marker (Hibiscus); green marker (Neem); +ve control (Tetracycline disc - antibiotic); -ve control (Ethanol disc).
- With sterilized tweezers, place an equal amount of tabs in each extract, ethanol, and antibiotics.

Preparation of Inoculums

- A loopful of the test organism *Enterobacter Sporosarcina Ureae* was inoculated into the nutrient agar plate(criss-cross lines across the nutrient agar plate so that it is covered with the test organism). (10 plates)

- Once the Petri Dish is inoculated, use the tweezers to place each type of tab into one of the four quadrants.
 - Next, place one ethanol disc in the center of the quadrant of each media plate where it is marked to show -ve control.
 - Place one tetracycline disc in the center of the quadrant of each media plate where it is marked to show +ve control.
 - Place one hibiscus extract disc in the center of the quadrant of each media plate, which is marked in red.
 - Place one neem extract disc in the center of the quadrant of each media plate, which is marked in green.
- Flip the prepared media plates so that the disk with condensation is on top.
- Store at room temperature (71 deg f) for 5 - 6 days.

3. Variables

3.1. Constants

- *Sporosarcina Ureae*, Agar, Slant(1) - Bacteria type 1
- Nutrient Agar, Prepared Media Plates, 100 x 15 mm, Pack of 10 (2 packs)
- Enterobacter aerogenes, Living, Nutrient Agar, Slant (1) - Bacteria type 2
- Economy Antibiotic Disk, Tetracycline, 30 mcg(1)

Tools

- Loop Swab (1)
- Box of Sterile Gloves (1)
- Tape
- Black Sharpie
- Red Sharpie

PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

- Green Sharpie
- Lighter (For sterilization)
- Metal Tweezers
- Paper Tabs (100)

3.2. Controls

- Positive Control - Tetracycline (Antibiotics)
- Negative Control - Ethanol

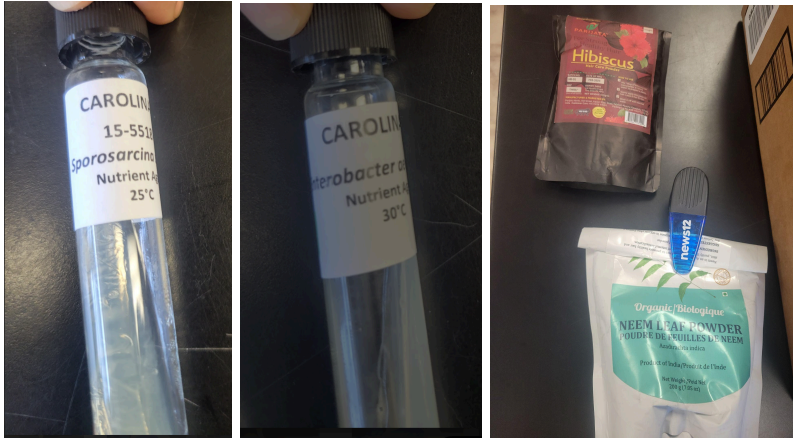
3.3. Independent Variable

The independent variables are the Hibiscus and Neem extract tabs placed into the petri dishes.

3.4. Dependent Variable

The dependent variables are the zones of inhibition of the bacteria.

4. Pictures



4.1. Data

Legend:

For the purposes of analysis - I will use a likert scale ranging from 0-3 where

0/. (zero) represents - no evidence of bacteria;

1 is some evidence of bacteria;

2 is evidence of bacteria;




3 is most evidence of bacteria.

Negative control(Ethanol)

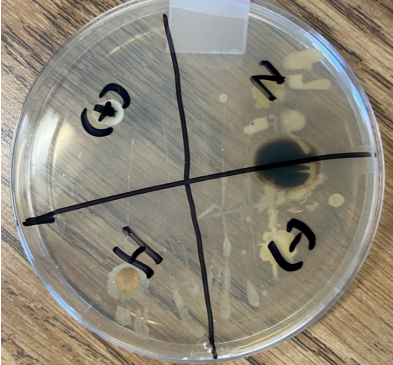
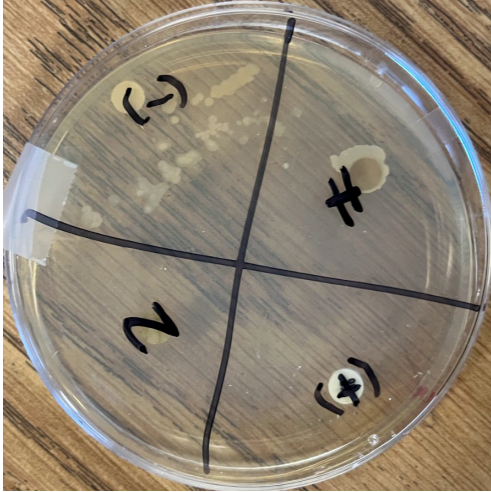
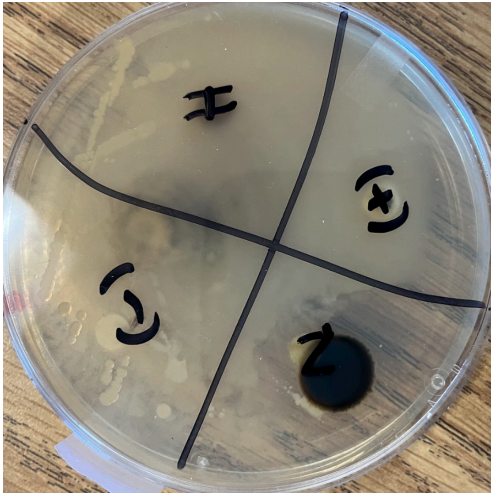
Positive control - Tetracycline

<u>Plate ID</u>	<u>+ve</u>	<u>-ve</u>	<u>Hibis cus</u>	<u>Neem</u>	<u>Comments</u>




PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

	<p><u>1</u></p>	<p><u>2</u></p>	<p><u>2</u></p>	<p><u>3</u></p>	
	<p><u>0</u></p>	<p><u>3</u></p>	<p><u>0</u></p>	<p><u>3</u></p>	<p>Condensation on the Petri Dish</p>
	<p><u>0</u></p>	<p><u>3</u></p>	<p><u>1</u></p>	<p><u>2</u></p>	<p>Condensation on the Petri Dish</p>

PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

	<u>0</u>	<u>3</u>	<u>1</u>	<u>2</u>	Black Spot on the border of N and -ve is mold.
	<u>0</u>	<u>3</u>	<u>1</u>	<u>0</u>	
	<u>0</u>	<u>3</u>	<u>1</u>	<u>0</u>	Multiple mold spots and a significant amount of condensation

PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

	<u>0</u>	<u>3</u>	<u>1</u>	<u>1</u>	
	<u>0</u>	<u>3</u>	<u>1</u>	<u>2</u>	Bacteria is much more developed than other tests
	<u>0</u>	<u>3</u>	<u>3</u>	<u>3</u>	

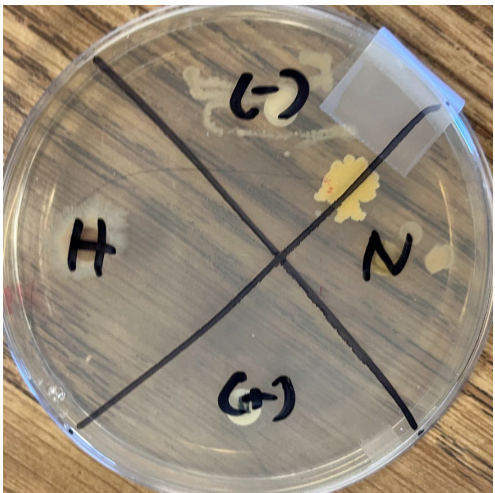


PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

	<u>0</u>	<u>2</u>	<u>2</u>	<u>0</u>	
	<u>1</u>	<u>3</u>	<u>1</u>	<u>3</u>	
	<u>0</u>	<u>1</u>	<u>0</u>	<u>1</u>	<p>Condensation across the dish and white cavities appearing, high chance of mold.</p>

PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

	<u>0</u>	<u>1</u>	<u>0</u>	<u>1</u>	
	<u>0</u>	<u>3</u>	<u>2</u>	<u>0</u>	
	<u>0</u>	<u>2</u>	<u>3</u>	<u>1</u>	

PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

	<u>0</u>	<u>2</u>	<u>1</u>	<u>1</u>	Yellowish white cavity appearing, high chance of developed bacteria or mold
	<u>1</u>	<u>3</u>	<u>2</u>	<u>3</u>	
	<u>1</u>	<u>3</u>	<u>3</u>	<u>3</u>	

	<u>2</u>	<u>3</u>	<u>3</u>	<u>1</u>	
	<u>3</u>	<u>0</u>	<u>3</u>	<u>0</u>	Error

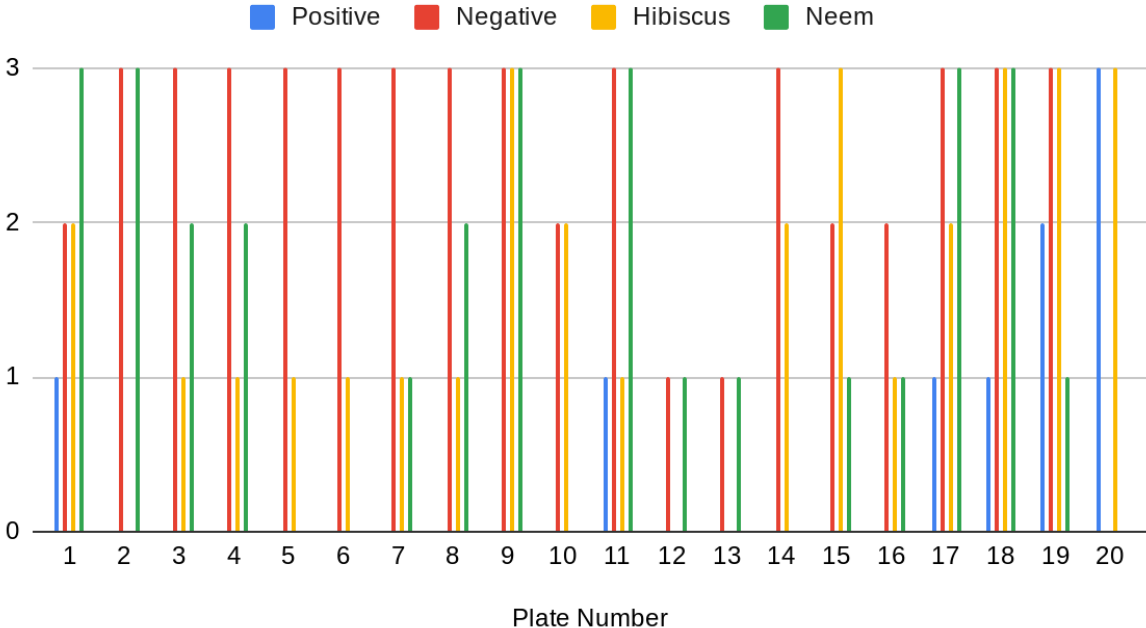
Incubated Petri Dishes

As shown above, there are twenty plates in total, where *Sporosarcina Uraeae* was inoculated into the nutrient agar plate into ten plates and Enterobacter was inoculated into the nutrient agar plate into ten additional plates. These photos were taken ten days after the inoculations. The quadrants marked negative (-ve) show bacteria (criss cross inoculation), and the quadrants marked H (Hibiscus extract) and N (Neem extract) and positive (+ve) show little if not no evidence of bacteria. The blotches on some plates

indicate mold development. The data was measured using the growth inhibition of the bacteria. 0 represents no signs of bacteria, 1 represents little signs of bacteria, 2 represents signs of bacteria, and 3 represents heavy signs of bacteria. The ethanolic negative control had an average growth inhibition (GI) of 2.45, while the antibiotic positive control had an average GI of 0.45. Both Hibiscus and Neem had an average GI of 1.5.

4.2. Chart

Inhibition Test Results



5. **Discussion/Analysis**

From the data, we can see that the negative control and positive both reacted in the opposite way, which gives some credibility to our tests. For the most part, this study validates prior studies (Abass, 2021)(Abdallah, 2016)(Al Sheik, 2020)(Chedid, 2014)(Da-Costa-Rocha, 2014)(M. Hoque, 2007)(Sandhir, 2021), all of which indicated that plant based antibacterial substances are as potent as antibiotics. In this study, this is evidenced in plates 2,4,5,6,7,8,12,13,14,16, where both the Hibiscus and Neem quadrants have the same effect as the positive control against (1) Enterobacteria and (2) *Sporosarcina Ureae*. While some plates exhibit mold (4 and 6), and some other plates show the bacteria streaks, these inconsistencies can be attributed to novice effort in inoculation. The results clearly indicate that Hibiscus and Neem contain the required phytochemicals to be used as an alternative to antibiotics in combating drug-resistant bacteria.

6. **Limitations of Research**

The limitations of research are that this study was conducted and experimented with novice researchers, which had an effect on parts of the study such as the inoculation process. This resulted in not all the quadrants receiving the same amount of bacteria, which compromises the accuracy of antibacterial growth as observed after incubation.

7. **Errors**

The plates were not separated by the type of bacteria being tested. This limits a more specific analysis of the antibacterial properties of Hibiscus and Neem.

8. **Conclusions**

In conclusion, *Hibiscus Sabdariffa* (Hibiscus) and *Azadirachta Indica* (Neem) are both effective Plant Derived Substances that can be used as antibacterial solutions, which are Tetracycline (Antibiotic). Tetracycline, while shown to have a slightly more effective inhibition effect, is artificial and has many adverse side effects, such as the compromisation of the gut microbiome, which is linked to multiple diseases, deficiencies, and cancers. Hibiscus and Neem as a natural alternative do not have these severe side effects, while balancing the homeostasis in the body, protecting the body from diseases and deficiencies.

9. **Acknowledgements**

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Kirby Bauer Disc Diffusion Method (01/04/2025)

This method involves the diffusion of antimicrobial agents from a paper disc into an agar medium that has been inoculated with the test microorganism.


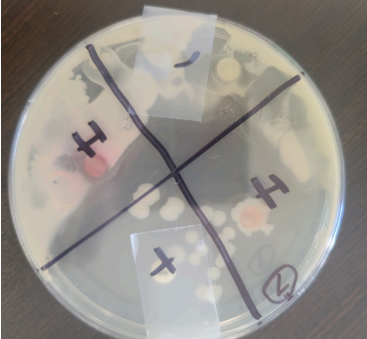

In this case, the sterile blotting paper disc (10 mm) was soaked in the diluted ethanol extracts in such a concentration that the amount of solution absorbed by each disc was 5 mg of each extract of herbal preparations (Hibiscus Sinensis).

Preparation of Inoculums

- A pipette full (5 ml) of the test organism *Staphylococcus Epidermis* was inoculated into the nutrient agar plate
- Once the Petri Dish is inoculated, use the tweezers to place each type of tab into one of the four quadrants.
 - Next, place one ethanol disc in the center of the quadrant of each media plate where it is marked to show -ve control.
 - Place one tetracycline disc in the center of the quadrant of each media plate where it is marked to show +ve control.
 - Place one Hibiscus extract disc in the center of the quadrant of each media plate, which is marked in red.
 -
- Flip the prepared media plates so that the disk with condensation is on top.
- Place the prepared media plates on a heating pad that was set at 71 deg f for 5 - 6 days.

Pictures

PLANT BASED PHYTOCHEMICALS EFFECT TO DRUG RESISTANT BACTERIA

<u>Plate ID</u>	<u>+ve</u>	<u>-ve</u>	<u>Hibiscus</u>	<u>Comments</u>
	<u>1</u>	<u>2</u>	<u>2</u>	
				
				
				

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