

Pilot And Proposal For Assessment Of Alternative Treatment Of Corneal Ulcers Without Emergence Of Resistance And Recurrence

Miss. Theertha,
Student, University High School, Irvine, California.

Dr. Prabhu Venkatesan,
Associate Professor Ophthalmology, Avmc, Pondicherry.

Dr. Charanya, M.S.
Post-Graduate, Avmc, Pondicherry.

Dr. P. Mishra,
Professor And Hod, Ophthalmology, Avmc, Pondicherry.

Abstract

During the summer of 2022, I visited rural India and witnessed many diseases being treated with neem and turmeric. I wondered how effective these methods really were. This led me to my research question: "What are the effects of turmeric extract and neem oil on bacterial growth?" First, my team used cotton swabs to collect conjunctival samples from individuals with conjunctivitis, then used these swabs to streak three plates prepared with Mueller Hinton Agar. These plates were incubated at 37 degrees Celsius for 48 hours and observed for bacterial growth. Once bacterial growth existed, the bacteria was gram-stained. Then, turmeric extract was introduced onto plate number 2, and neem oil was introduced onto plate number 3. Plate one was the control plate. All plates were incubated at 37 degrees Celsius for 24 hours and observed for bacterial growth inhibition. After 48 hours, bacterial growth on the agar plates from conjunctival swabs were present. After turmeric extract was added to the bacterial growth, there were very clear sites of bacterial growth inhibition on the petri dish. These areas showed a white ring around the bacterial colonies which showed that growth of the colonies was being inhibited. My hypothesis was ultimately correct, because I predicted that there would be a lot of inhibition of bacterial growth in the petri dish with turmeric used.

This is due to the many compounds in turmeric, including Curcumin and many more. These compounds are all known for being effective antibacterial, antifungal, and anti-inflammatory agents.

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I. Introduction

Every day we see and treat so many cases of corneal ulcers, which are caused usually by common pathogens like bacteria, viruses, fungi or Acanthamoeba. We have many antibiotics, antifungals, antivirals and other effective drugs. While they are very effective in the treatment of ulcers, they also have some disadvantages. One main disadvantage is that these antimicrobials are often specific to a particular pathogen, and due to this cannot be used when there is a superadded infection involving multiple pathogens, such as mixed infections with bacteria and fungus. This issue results in the need to use multiple drugs [6].

Many rural Indian villages treat diseases such as smallpox and chicken pox with neem and turmeric. I was inspired by these old Indian medical treatments and wondered how effective these methods really were.

They had been used in India for the better part of two millennia, and I figured that, if utilized correctly, neem and turmeric extracts may eventually be able to be used in modern medicines to make them more effective.

Turmeric is often referred to as yellow gold, and the Sanskrit name for neem is "arishtha." meaning "reliever of sickness. Both these herbs are known to be powerful healing agents and antibacterials. Neem is a powerful antibacterial that can kill both gram-negative and gram-positive bacteria. This ability is attributed mainly to nimbidin, a compound found in neem extract and contributes to its antibacterial properties [1]. Neem contains a limonoid, which is a natural compound found in citrus fruits and other plants, called nimbin, that allows it to have antibacterial properties.

Although nimbin alone doesn't demonstrate any strong antibacterial activity, when combined with other components, like it is in a natural neem tree extract, it contributes greatly to the overall effectiveness against bacteria. Likewise, neem tree extract without nimbin also doesn't demonstrate any strong antifungal activity. Overall, the combined effect of various compounds in a neem leaf extract, rather than any single compound like nimbin, is responsible for neem's strong antibacterial properties [2].

Turmeric contains curcumin, which is a bright yellow chemical compound [3]. Turmeric's anti-inflammatory, antifungal, and antibacterial properties are both attributed to curcumin.

Curcumin is an anti-inflammatory agent because it targets specific molecules and pathways that control the cell cycle. It also blocks the two enzymes mainly associated with inflammation [4].

Additionally, curcumin is also a good antibacterial because it is capable of damaging bacterial cell membranes by binding to the bacterium's cell wall or a protein associated with a bacteria [5].

It can damage the permeability and integrity of bacterial cell membranes in Gram-positive and negative bacteria, which leads to bacterial cell death.

Curcumin is also capable of targeting DNA in bacteria. This background information enabled me to start my experiment with a solid idea of what I was doing.

Problem Statement

The most common side effect of the synthetic antibiotics, is the emergence of resistant strains, which always accompanies the use of these synthetic antibiotics. If the corneal ulcer extends to the basement membrane and stroma, there is always the formation of scar tissue, which leads to severe visual impairment depending on the area involved [6].

Aim Of Study/Research Question

The aim of this study is to explore the use of alternative ayurvedic medicines which are effective to use against most common pathogens so that one drug alone is used against mixed infections. The drug should not produce resistant strains, and should be effective without causing major side effects. This is why ayurvedic drugs came to mind. We thought of using a combination of natural substances, which are effective in suppressing most common pathogens without producing resistant strains, and also reduce the formation of scar tissue.

We would like to find the extent to which turmeric extract and neem oil inhibit bacterial growth due to the compounds such as curcumin and nimonol present in them.

Brief Description Of Research Work Planned

We plan to conduct this experiment in vitro on the culture plate in which the pathogen, which is taken from the discharge from the corneal ulcer patients, is allowed to grow. After this, we will expose the pathogen separately to both turmeric extract and neem oil, and assess the efficacy with which pathogen growth is inhibited.

We would like to assess the recurrence rate of infection after the ayurvedic treatment. Lastly, we also want to assess the extent of scar formation and incidence of perforation in cases of fungal corneal ulcer when the patients are put on ayurvedic treatment after the efficacy is arrived at.

II. Methodologies

We intend to collect samples from corneal ulcer patients with no preference as to the pathogenic aetiology. We will take the exudate from the eye and culture it in vitro which serves the purpose of identifying the organism and also to check the sensitivity. Then we will do a control study with the necessary antibiotics that are suggested routinely for that condition and see the clearance of the growth.

In another culture which is inoculated with the same exudate, we plan to subject the to the clearance area assessment with the mixture of neem oil and turmeric extract. The molecular change that may be producing resistance is also to be measured.

The efficacy and safety of the ayurvedic drug on multiple aetiology is also noted instead of using different drugs for different pathogens.

Our aim is to know whether this extract is equally efficacious on all different pathogens without causing any resistance or allow the scar formation.

The first step in this experiment was to wear gloves and ensure that the lab environment was sterile. We used sterile cotton swabs to gently collect conjunctival swab samples from individuals with conjunctivitis. We streaked three petri dishes prepared with Mueller Hinton Agar. Then, we incubated the plates at 37 degrees Celsius for 48 hours and observed for bacterial growth. We gram stained the bacterial colonies and found the bacteria is found to be gram negative, then introduced 1 milligram of turmeric mixed in sterile water into plate number 2, and introduced 10 microliters of neem oil into plate number 3. We left plate number 1 alone as it was to be the control group. Then we incubated all three plates, including the control at 37 degrees Celsius for 24

hours and observed for signs of growth inhibition.

Inclusion Criteria

1. Patients with corneal ulcer due to any aetiology.
2. Patients with corneal ulcer with systemic diseases like diabetes mellitus.

Exclusion Criteria

1. Patients already on synthetic antibiotics.
2. Patients who are in the paediatric age group.

Statistical Analysis

This is a prospective in-vitro study of the exudates taken from the patients who are suffering from corneal ulcer after informed consent obtained from the patients.

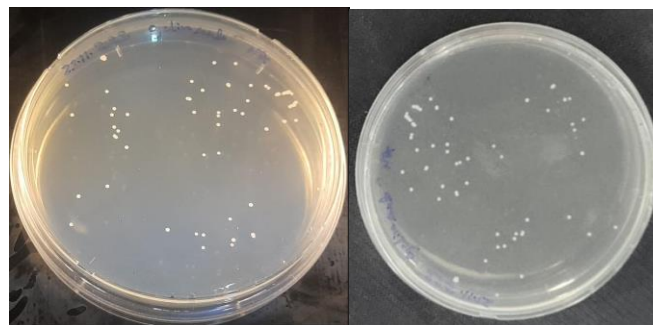
We are collecting the exudate from the patients who have corneal ulcers and not on any antibiotics and the exudate is cultured in a nutrient solution and the pathogen is identified. Whether the pathogen is bacterial, viral, fungal or acanthamoeba it is identified and grown in different media and then neem and turmeric extract is added in each individual culture plate and then the clearance is studied, to assess the efficacy. In the meantime, the identification of the pathogen from the culture is dictating the standard treatment protocol that is going to be followed in the patient, as per conventional treatment regime.

Prognosis is determined by the SOP and this experimental drug is not given directly to the patient or tried for the treatment with the patient. Only the effect of the drug is assessed in vitro on the clearance made by the drug on the culture plate and we can study whether this extract is effective in bacterial population or on viral population and also on the pathogen, whether it is effective on mixed aetiology or only on specific pathogen.

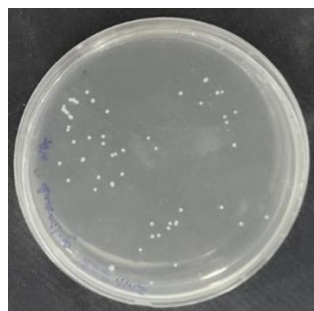
III. Results



Inoculation of Sample onto Growth Medium



(both pictures above) Initial Bacterial Growth before introduction of neem and turmeric. Bacteria colonies may be Clostridium. Gram negative



Initial Bacterial Growth before introduction of neem and turmeric



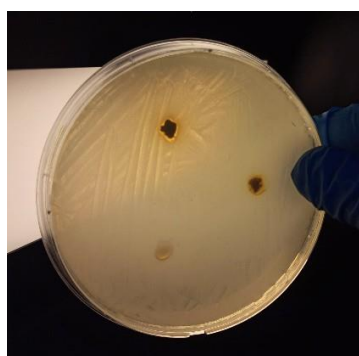
Gram-Staining result - Bacterial colonies were found to be Gram Negative. Based on colony shape and size, the bacteria is most likely *Klebsiella Pneumoniae*



Preparation of Turmeric Antibacterial Activity Assay



Neem oil placed on bacterial growth



Results of Turmeric antibacterial activity assay

After 48 hours, there was the presence of significant bacterial growth on nutrient agar plates from conjunctival swabs. There was no visible change in the bacterial growth of the neem petri dish. This could be due to the incorrect concentration of neem extract present in the commercially produced neem oil that we initially used for this experiment. After turmeric extract was added to the bacterial growth, there were very clear sites of bacterial growth inhibition on the petri dish. These areas showed a white ring around the bacterial colonies which showed that growth of the colonies was being inhibited.

IV. Discussion

There was significant bacterial growth in all petri dishes after 48 hours. While the petri dish with neem used as an antibacterial did not work, possibly due to the quality of neem oil used, the petri dish with turmeric used as an antibacterial showed noticeable zones of inhibition of bacterial growth, indicating turmeric's

effectiveness as an antimicrobial.

Variables that could have influenced the results include the unintentional (small) fluctuation of temperature, as this experiment was conducted in a lab used by the entire college. Additionally, the neem oil used in this experiment initially, as stated before, was not of sufficient quality because it was not certified for lab use. This led to an unnoticeable/nonexistent result in the experiment even though neem is already a proven antibacterial.

In agreement with our hypothesis, the turmeric petri dish did exhibit significant inhibition of bacterial growth. This is due to the many compounds in turmeric, including but not limited to Curcumin, Demethoxycurcumin,

Bisdemethoxycurcumin, Ar-turmerone, α -turmerone, and β -turmerone. These compounds are all known for being effective antibacterial, antifungal, and anti-inflammatory agents. The reason we got the results that we did was because curcumin targets specific molecules and pathways that control the cell cycle, and blocks inflammatory cytokines and enzymes, including cyclooxygenase-2 (COX-2).

Since turmeric proved to be an effective antimicrobial agent, it is a possibility for turmeric to be used in the future in an antifungal or antibacterial medication. Our project did turn out as we expected, because we predicted that there would be a lot of inhibition of bacterial growth in the petri dish with turmeric used. It did not turn out as we expected with the neem oil, however. This is because, scientifically, neem was supposed to have the same if not greater inhibitory effect as turmeric on the bacteria.

While curcumin is an effective antimicrobial, its poor bioavailability, which is the proportion or amount of a substance or drug that is able to reach its intended site of action within the body, is the main reason it isn't used in any formulated drugs today [8]. A further research question of ours would be to figure out how to format or present curcumin in a drug so that it works effectively on human patients.

This project brings to light the fact that, while a lot of parts of olden culture may not be relevant today, there is a lot we can still learn from ancient medicine that would help with a lot of our world's health problems. Many parts of part of Indian culture, including food, have some significance and benefit to health. These health benefits may be more impactful than previously realized, and certain herbs and ingredients such as turmeric and neem could be used or translated into modern medicine.

If we had a chance to do this project again or expand on it, we would make sure to find higher quality neem oil to use during the experiment. This is because we were looking forward to comparing and contrasting the inhibitory effects of neem oil and turmeric on bacterial growth. However, we were not able to because the neem oil that we used was not highly concentrated enough to actually retain its antibacterial properties.

Our next steps for researching this topic would be to figure out if it is possible to use curcumin in Western/modern medicine. If it does prove possible, we would like to come up with a way to increase the bioavailability of curcumin so that it would actually function effectively as a medication without exceeding FDA guidelines. These results could be applied in everyday life because this could turn into the next major antifungal or antibiotic. Similar to how sick children in rural India are treated in turmeric and neem, these herbs could be utilized in western medicine to better millions of lives.