

Isolation, identification and characterization of bacteria and antibacterial susceptibility of bacteria with different plant leaf extracts.

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Abstract

The growth of microorganisms in our environment (bacteria, viruses, algae, fungi and protozoa) are pervasive, influential and can increase the toxicity in our surrounding, which can be harmful for the human body and can be life threatening at times. Bacteria like Gram positive and Gram negative bacteria which are sensitive to many drugs are already in use but these bacteria are becoming harmful because of the resistance provided by their outer membrane. So, to have an outcome friendly to nature, research was done on different plant leaves. Main purpose of research was to assess the efficacy of Jamun leaf extract (*Syzygium cumini*), Guava leaf extract (*Psidium guajava*), and Lemon leaf extract (*Citrus lemon*) against two gram negative bacteria (*Proteus mirabilis*, *Staphylococcus epidermidis*) and two gram positive bacteria (*Corynebacterium xerosis*, *Bacillus megaterium*) which are some of soil borne and water borne bacteria. The leaves were extracted in three different solvents like Acetone, hexane and methanol. After that the efficacy of these extracts with concentration of 50µl and 100µl were tested against those bacteria through well diffusion method and disc diffusion method and Zone of inhibition (ZOI) was measured. According to the findings of antibacterial assay, Jamun extract showed antibacterial activity against all bacteria in both concentrations acting as natural antibacterial agent followed by Guava extract. Lemon extract showed minimal zone of inhibition against all the bacteria. This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological and medicinal properties. This study may provide useful insight for next level natural treatment strategies and for further researches in this study.

Key words = Antimicrobial activity, Plant extract, Well diffusion method, Gram positive bacteria and Gram negative bacteria, Methanol, Acetone, Hexane.

Introduction

Isolation, identification and antibacterial susceptibility of bacteria with different plant leaf extract is an area of research that looks at how different plant leaf extracts can be used to identify and isolate bacteria, as well as to determine their susceptibility to antibacterial agents. This research is particularly relevant to the medical sciences, as it can be used to study the effects of natural remedies on bacteria and to help identify and develop new treatments for bacterial infections. By isolating, identifying and determining the susceptibility of bacteria to different plant leaf extracts, researchers can gain important insights into the mechanisms of action of these natural remedies and the potential for their use in treating bacterial infections. This research can also help to identify new targets for antibacterial agents, which can be used to develop more effective treatments for bacterial infection.

Antibacterial resistance is a natural biological phenomena exacerbated by mien of drugs. The problem of Microbial Resistance is growing and the outlook of the wise 9 antimicrobial drugs in future is still uncertain. •Therefore action must be taken to reduce this problem, for example to control the use of antibiotics, to develop research to better understand the genetic mechanism. Resistance and to continue studies to develops new things either synthetic or natural. A long period of time, plant have been a Variable source “natural products for maintaining human health. Hooking back on the history of human diseases, infection have accounted for a proportion of Disease as a whole.

The first antimicrobial agent in the would was salvarsan, a vremedy for syphilis that was Synthesized by Ehrlich in 1910. In 1935, Sulfonamides were developed by Domagh and researchers. These drugs were Synthesized by compounds and had limitations in 4 terms of and efficacy... In 1928, Fleming, discovered penicillin.. He found that the growth of *staphylococcus*

aureus inhibited in a zone surroundings, blue mold (a fungus from the *Penicillium* genus) in a contaminated. culture dishes, leading to the finding that a microorganism would produce substance that could the growth of ether microorganisms.

Methanol is a good solvent for extraction and it is frequently used in biology because of its polarity. It is capable of extracting both lipophilic and hydrophilic molecules or substances. The other advantage is that it be removed easily at room temperature because it is highly volatile..

LEAVES OF ANTIBACTERIAL PLANTS

- JAMUN LEAF (*Syzygium cumini*)
- GUAVA LEAF(*psidium gujava*)
- Lemon leaf

USE OF BACTERIA

- *Staphylococcus epidermidis*
- *Corynebacterium xerosis*
- *Bacillus megaterium*
- *Proteus mirabilis*

MATERIALS AND METHODS

SAMPLE

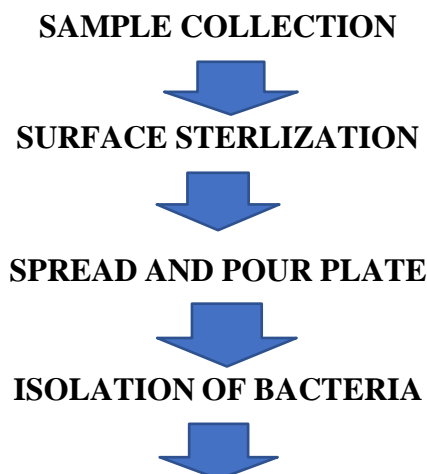
Petrol pump soil sample

NUTRIENT MEDIA

. Nutrient Agar media

Nutrient Broth

Manitol Media



CHARACTERIZATION OF BACTERIA



ANTIBACTERIAL PROPERTIES OF BACTERIA

SAMPLE PROCESSING

After collecting the samples, pH was measured and after that using pour plate, spread plate method or streaking method. Some of the processes are:

PH measurement: - After rinsing the pH meter with distilled water it was dipped into the beaker poured with juice sample. After 30 seconds the reading of the pH meter was noted down and the pH meter was rinsed with distilled water and ethanol, dried and switched off. The process was followed for next all samples.

Serial dilution: -Made dilutions of saline soil sample up to 10^{-3}

Pour plate method for bacterial culturing: 1 ml of saline soil sample were taken and added to different petri plates, then added nutrient agar media, this method is known as pour plate method given by Robert Koch

Different bacterial colonies were observed next day. Picked colony from same culture plate and proceed it for gram staining.

Gram staining

Pick Different bacterial colonies from culture media unstained it using gram staining method.

Gram staining method contains four different stains which are crystal violet, iodine, 70% alcohol and safranin.

The timing for staining is 30 seconds for crystal Violet, 1 minute for iodine, 30 seconds for alcohol and 1minute for safranin.

1. Crystal Violet (primary stain)
2. iodine solution (mordant that fixes crystal Violet to cell wall)
3. 70% alcohol (decolorizer)
4. Safranin (secondary stain)

RESULTS AND CONCLUSION

The herbal extract of Syzygium cumini leaves prepared with methanol, hexane and acetone has shown zone of inhibition (ZOI) with all bacteria. The maximum zone of inhibition was seen in Proteus mirabilis (Acetone extract).

The herbal extract of Citrus limon leaves prepared with methanol, hexane and acetone has shown maximum ZOI with Corynebacterium xerosis and Proteus mirabilis (Acetone extract). The minimum ZOI was seen with staphylococcus epidermidis and Bacillus megaterium (Methanol extract).

The herbal extract of Psidium guajava leaves prepared with methanol, hexane and acetone has shown zone of inhibition (ZOI) with all bacteria except in Bacillus subtilis (Methanol extract). The maximum zone of inhibition was seen in Proteus mirabilis and Corynebacterium xerosis (Methanol extract)

ANTIBACTERIAL SUSCEPTIBILITY OF BACTERIA

- Drying and grinding of leaves
- Leaf powder was placed in organic solvent for 48 hours Bacterial inoculation was done by swabbing method
- Nutrient agar was prepared and poured in plates
- Agar well diffusion methods is used to be screened for antibacterial activity
- Wells were made and extract were loaded into the wells at different concentration with the control and incubated for 24 hours
- Organic solvent evaporates after 48 hours
- Leaf powder was dissolved in DMSO
- Zones were analyzed and measured by zone measuring scale

OBSERVATIONS

Jamun leaf (*Syzygium cumini*)

Bacteria	Extracts			
	Methanol	Acetate	Hexane	Control
<i>Corynebacterium xerosis</i>	15	7	16	0
<i>Staphylococcus epidermidis</i>	14	5	10	0
<i>Bacillus megaterium</i>	17	14	16	0
<i>Proteus mirabilis</i>	17	18	9	0

Guava leaf (*psidium gujava*)

Bacteria	Extracts			
	Methanol	Acetone	Hexane	Control
<i>Corynebacterium xerosis</i>	16	0	9	0
<i>Staphylococcus epidermidis</i>	0	3	5	0
<i>Bacillus megaterium</i>	14	9	14	0
<i>Proteus mirabilis</i>	17	5	9	0

Lemon leaf (citrus lemon)

Bacteria	Extracts			
	Methanol	Acetone	Hexane	Control

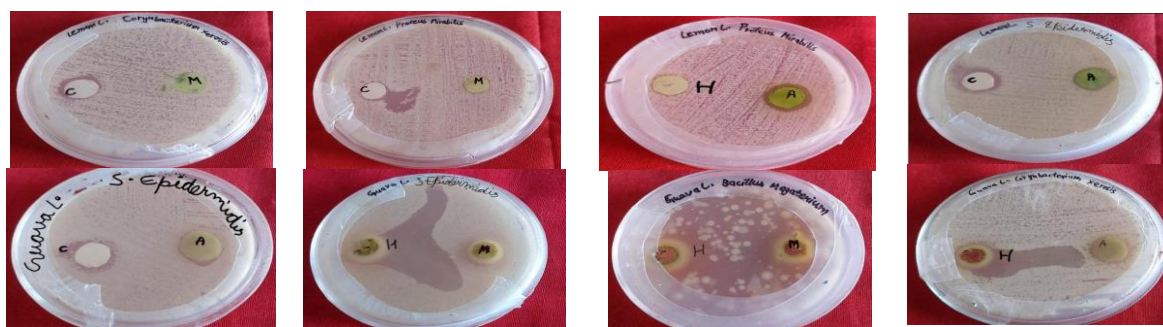
Corynebacterium xerosis	0	15	0	0
Staphylococcus epidermidis	5	10	12	0
Bacillus megaterium	7	6	0	0
Proteus mirabilis	0	14	0	0

Zone of Inhibition of Jamun leaf



Zone of Inhibition of Guava leaf

Zone of Inhibition of lemon leaf



a. The herbal extract of Syzygium cumini and Psidium guajava leaves prepared with hexane, methanol and acetone has shown zone of inhibition (ZOI) with all bacteria.

b. The herbal extract of Citrus lemon leaves prepared with hexane, methanol and acetone has shown maximum zone of inhibition (ZOI) only in Bacillus megaterium.

CONCLUSION

Among the three leaf extracts prepared with Syzygium cumini, Citrus limon and Psidium guajava leaves, the maximum inhibition was shown by Syzygium cumini leaf extract on staphylococcus epidermidis Bacillus megaterium, Proteus mirabilis and Corynebacterium xerosis followed by Psidium guajava and Citrus limon leaf extract.

Also the three leaf extracts prepared with Syzygium cumini, Psidium guajava and Citrus lemon the inhibition was shown by all the three leaf but the maximum ZOI was shown by Syzygium cumini on all bacteria.

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