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ANTI BACTERIAL ACTIVITY ON GRAM POSITIVE AND GRAM NEGATIVE BACTERIA USING GUAVA

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ABSTRACT

To determine the anti bacterial potential activity of guava(psidium guajava) leaf extracts against gram positive(staphylococcus aureus) and negative bacteria (E.coli) using methanol and isopropanol extracts showed inhibitory action. Only gram positive bacteria are susceptible to the two extracts, while other gram negative bacteria showed zone of inhibition. Major constituents in guava is quercetin,rich in flavonoids. In preliminary phyto chemical screening, the crude extracts gave the positive reactions for alkaloids, flavonoids, saponins, tannins. Significant of anti-bacterial activity was found by the extract of (isopropanol, methanol) of guava leaf extracts. It shows anti bacterial, anti diarrhoeal, anti spasmodic, anti inflammatory activity.

Keywords:*Staphylococcus aureus, E.coli, streptomycin, saline, Isopropanol, Methanol*.

1) INTRODUCTION

Herbal drugs in drug discovery
Natural products research continues to be important part of the drug discovery process. The main advantage of natural products as a source of lead compounds is the tremendous molecular diversity found in nature.

Safety and Toxicity of herbal medicines
The explanation of ―toxic‖ is ultimately a matter of viewpoint. Many ordinary foods contain constituents that could be allergic or considered as toxic such as the alpha gliadin produced by gluten in wheat, oats and rye, the cyanogenic glycosides in many fruit seeds, the thiocyanates of the Brassica vegetables, alkaloids of the Solanaceae and lectins of many pulses including soya and red kidney beans.

Bacteria:
Some bacteria live in very salty water, like that of the Dead Sea. One type of bacteria lives in water that drains from coal mines, which is extremely acidic at a pH of 1.

Types of Bacteria:
Based up on the Gram staining technique developed by Hans Christian Gram.The bacteria are classified into two types:

Gram positive bacteria:
They have a large peptidoglycan structure. As noted above, this accounts for the differential staining with Gram stain. Some Gram positive bacteria are also capable of forming spores under stressful environmental conditions such as when there is limited availability of carbon and nitrogen.
Spores therefore allow bacteria to survive exposure to extreme conditions and can lead to re-infection (e.g., pseudomembranous colitis from Clostridium difficile).

Gram negative bacteria:
They have a small peptidoglycan layer but have an additional membrane, the outer cytoplasmic membrane. This creates an

additional permeability barrier and results in the need for transport mechanisms across this membrane

2) LITERATURE REVIEW

Gislene et al., 2000 Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.

Madhuri and Pandey, 2009 Phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activity.

Vieira et al. have also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of the *S. aureus*. Gnan and Demello testing guava leaf extract found good antimicrobial activity against nine different strains of *Staphylococcus aureus.*

3) AIM AND OBJECTIVES OF THE WORK

To determine the antimicrobial potential of guava (*Psidium guajava*) leaf extracts against two gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) which are some of foodborne and spoilage bacteria.

The aim of the present study is to determine the antimicrobial potential (*psidium guajava l*) leaf extracts the methanol and isopropanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts.

A board of organisms comprising 2 Gram-negative bacteria, *Escherichia coli (Escherichia coli , Living Bacteriophage Host, item no. 124300) and Salmonella enteritidis (Salmonella enteritidis, MicroKwik Culture, Pathogen, item no. 155350A), and 2 Gram-positive bacteria, Staphylococcus aureus (Staphylococcus aureus,coagulase positive),Culture, Pathogen, item no. 155554A) and Bacillus subtilis (Bacillus subtilis,Living,* item no. 154872) was selected to test the guava extracts have ability to inhibit the growth

4) PLAN OF WORK:

Firstly check the review of literature then collection of materials then procurement of prepared psidium guajava extract later drying of the extract their phytochemical screening is done after that bacterial culture is prepared and finally evaluation of anti bacterial activity.

5) MATERIALS AND METHODS:

a) Media, chemicals and equipments

b) List of test organism

c) Standard drug profile

d) Extraction of *psidium guajava.l*

e) Phytochemical analysis of pisdium guajava.L

f) Collection of extract

g) Pure culture by cup plate method

h) Zone of inhibition

Media and chemicals

S.No.	Name Of Chemicals
1.	Chloroform
2.	Methanol
3.	Isopropanol

4.	Yeast
5.	Agar
6.	Peptone
7.	Sodium chloride
8.	Saline
9.	Distilled water
10.	Streptomycin
11.	Nutrient broth
12.	ethanol

List of equipments

S.No	Name Of Equipment
1.	Hot air oven
2.	Laminar air flow
3.	Refrigerator
4.	Incubator
5.	Micropipette
6.	Inoculation loop
7.	Soxhlet apparatus
8.	Autoclave

List of organisms

S.No	Test Of Organisms
1.	*Bacillus subtilis*
2.	*Staphylococcus aureus*
3.	*Escherichia coli*
4.	Pseudomonas aeroginosa

Standard drug profile

Name : STREPTOMYCIN

Chemical Names: Streptomycin; Streptomycin A; Streptomycin sulphate; Streptomycin sulfate;

Mechanism of action :

Streptomycin is a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit.
This results in an unstable ribosomal-mRNA complex, leading to a frameshift mutation and defective protein synthesis; leading to cell death.

Pharmacology:
Streptomycin is an aminoglycoside antibiotic derived from *Streptomyces griseus* with antibacterial activity.
Streptomycin is an aminoglycoside antibiotic. Aminoglycosides work by binding to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, leaving the bacterium unable to synthesize proteins vital to its growth.
Aminoglycosides are useful primarily in infections involving aerobic, Gram-negative bacteria, such as *Pseudomonas, Acetobacter, and Enterobacter.*
Aminoglycosides are mostly ineffective against anaerobic bacteria, fungi and viruses.

Method of **Preparation of the extract (Soxhletion) :**
The aim of this activity is to investigate the antimicrobial effects of plant material after extracting compounds using the relatively simple Soxhlet method .
The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor.
The side arm is lagged with glass wool. The solvent is heated using the heating mantle and will begin to evaporate, moving through the apparatus to the condenser.
The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again.
The process should run for a total of 24 hours. Once the student has set up the extraction it can be left to run without direct supervision.

It is not advised to leave the equipment completely alone due to the mix of running water and an electrical appliance, so a technician or other lab user should be made aware.

The equipment can be turned on and off when overnight running is not permitted, and the time split over a number of days. This could be plant material that has no known antimicrobial effect at the testing stage.

e) PHYTOCHEMICAL STUDIES AND ANALYSIS:

The major constituents of phytochemical consist of carbohydrates, aminoacids, proteins, and chlorophylls, while, secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins, etc .

Flavonoids were present in chloroform and benzene extracts. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. Guava fruit contain high vitamin _C' than citrus fruits and also appreciable amounts of vitamin A as well

Test for Alkaloids

Mayer's Reagent:

To 1ml of the extract, 2ml of Mayer's reagent was added. Appearance of dull white precipitate indicated the presence of alkaloids.

Test for Flavonoids:

To 1ml of extract, 1ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids.

Test for Steroids Lieberman-Burchard's Test:

The extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

Different activities shown:

Anti-bacterial activity

The extract also showed in vitro antimicrobial activity against e.coli, salmonella typhi , staphylococcus aureus. Proteus mirabilis, and shigella dysenteria. Another paper showed the effectiveness of the leaf extract against staphylococcus aureus .

Bark tincture exhibited higher efficacy in controlling the mycelia growth of dermatophytes than the leaf tincture .The tincture showed fungicidal property in different concentrations but exhibited only fungistatic property in different concentrations but exhibited only fungistatic property in case of C.albicans .

Anti-diarrhoeal activity

A leaf infusion is taken in Ghana and Nigeria for stomach complaints e.g.: constipation , and in Admawa with red potash for dysentery ; a decoction is taken in Senegal to combat diarrhea and dysentery ;the shoots may also be used , while in neighboring .

The ripe fruit is a good appearance , and should be eaten with the skin , for without it , costiveness results . The unripe fruit is said to in digestible , causing vomiting and feverishness , but it is sometimes employed for diarrhoea.

f) COLLECTION OF EXTRACTS

Once the process has finished, the methanol, isopropanol, water should be evaporated using a rotary evaporator, leaving a small yield of extracted plant

material (about 2 to 3 ml) in the round bottom flask.

g) PURE CULTURE BY CUP PLATE METHOD

i. Preparation of test organism

a) Inoculum preparation

Bacterial inoculum preparation :

A pure colony of the test organism was taken using a sterile loop and transferred in to tubes having a sterile nutrient broth and incubated with shaking at 35 c -37˚c until the visible turbidity must equal the standard.

b) Maintenance of cultures:

The culture was maintained by sub culturing on to agar slants and incubated at 28˚c for about 7-10 days .After sufficient growth they are transferred in to production medium.

c) Composition of agar medium for 10 ml

Ingredients	**Gms / Litre**
Peptone	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

d) **Procedure:**

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

The following test organisms used in :

(a) Gram negative bacteria - *Escherichia coli* and *Pseudomonas Aerogenosa* .

(b) Gram positive bacteria - *Bacillus subtilis* and *Staphylococcus aureus* .

Diffusion assay or cup plate method

After extraction of extract compound from the solvent .Nutrient agar media was prepared and sterilized and inoculated within 24hrs culture of test organism and plated after solidification cups are made by a sterile rubber borer .

The compound concentrate was added in to the cup by micropipette and kept for diffusion of the compound and incubated at 37˚c for 24hrs and observe the zones of inhibition in the plates

ZONE OF INHIBITION

Then the zone of inhibition were measured after 24 hrs. of incubation at 37˚c for bacteria and 48hrs.

Minimum inhibitory concentration:

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

6) Results:

a) Sterility testing of Plant extracts

Methanolic and isporpanolic extracts of *pisidium gujava.l* was found to be free from bacterial as observed by streaking it on Nutrient agar plates after 2 days of incubation at room temperature.

b)cup plate method

After extraction of extract compound from the solvent .Nutrient agar media was prepared and sterilized and inoculated within 24hrs culture of test organism and plated after solidification cups are made by a sterile rubber borer.

The compound concentrate was added in to the cup by micropipette and kept for diffusion of the compound and incubated at 37˚c for 24hrs and observe the zones of inhibition in the plates.

The effectiveness of test and a range of antibiotics was determined against *bacillus*

subtilis, Staphylococcus aureus ,Esherichia coli ,and*Pseudomonas aerugenosa.*

In this technique, an inoculums bacteria was added to melted, cooled agar. The agar inoculum mixture is then poured into a petri dish.

Up on solidification, isolated cells are trapped within the agar matrix. These cells give rise to isolated pure colonies of the Bacteria.In an individual petriplate single sample was taken and the activity was compared by the minimum inhibitory concentration and zone of inhibition studies.

c) Evaluation of activity

Minimum inhibitory concentration:

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

Zone of Inhibition (Kirby-Bauer test):

In this, the size of the zone of inhibition indicates the degree of sensitivity of bacteria to a drug. In general, a bigger area of bacteria-free media surrounding an antibiotic disk means the bacteria are more sensitive to the drug the disk contains.

Against to both gram positive and gram negative bacteria.

As shown in the table below the different zone of inhibitions of both the aqueous and methanolic and ispropanol extract, streptomycin were given.

CONCLUSIONS

These studies conducted on leaf extracts of *psidium guajava.L* on gram negaitive bacteria and gram positive bacteria are included in this thesis. In this book the author descrided the importance of drug from natural origin , a review of the the literature on the plant and the previous work and biological work was also given.

In the preliminary phytochemical screening, the curde exyract gave the positive reaction for alkaloids, flavonoids, saponins, tannins, proteins, etc. significant of anti-bacterial activity was found by the exact of (isopropanol , methanol) of guava leaf extracts.

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