

*Development of Antimicrobial
Bio-sheets: Crude Medicinal
Plant Extracts and Nanoparticle
Synthesis for Advanced
Packaging*

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INTRODUCTION

Food contamination is a major concern in the food supply chain, where microorganisms from the environment, handling surfaces, and packaging materials can compromise food safety and shelf life.

Commonly used packaging materials such as ;

- plastic bags
- foil covers
- natural leaves

can carry surface microbial loads that contribute to spoilage and transmission of food-borne pathogens.

•Frequent food-borne pathogens include; **Escherichia coli**, **Staphylococcus aureus**, **Salmonella spp.**, **Listeria monocytogenes**, **Pseudomonas spp.**, and various fungi that accelerate deterioration and pose health risks.





BIOSHEET-PLANT EXTRACT & NANOPARTICLE

- Rising consumer demand for minimally processed, chemical-free foods has driven the need for **active packaging systems** that not only store food but also actively inhibit microbial growth.
- Plant-based antimicrobial agents—rich in phytochemicals like phenols, flavonoids, and terpenoids—show strong inhibitory effects against a wide range of bacteria and fungi.
- Nanoparticle incorporation further enhances antimicrobial performance by increasing surface interaction and disrupting microbial cell structures.
- Developing a **biosheet coating** made from medicinal plant extracts and nanoparticle additives offers a promising approach to create an **advanced antimicrobial packaging layer** that reduces contamination on common food covers.

AIM

To develop an antimicrobial biosheet incorporating medicinal plant extracts, with the objective of reducing microbial contamination on food-packaging materials.

OBJECTIVES

- To evaluate the antimicrobial activity of crude medicinal plant extracts against common food-borne microorganisms.
- To synthesize nanoparticles using standard chemical methods and assess their antimicrobial efficacy.
- To formulate combinations of different components along with the plant extracts to determine enhanced or synergistic antimicrobial effects.
- To develop an antimicrobial biosheet incorporating the optimized formulation and assess its ability to reduce microbial load on common food-packaging surfaces.



Materials and methodologies

1. PREPARATION OF MEDICINAL PLANT CRUDE EXTRACT



Ginger
(Zingiber officinale)



Common Rue
(Ruta graveolens)



Neem
(Azadirachta indica)



Tulsi
(Ocimum tenuiflorum)

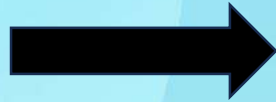


Mint (Mentha sp.)

PROCEDURE



Grind the plant material



Boil the mixture



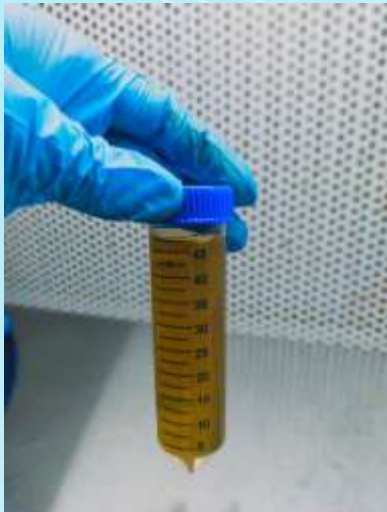
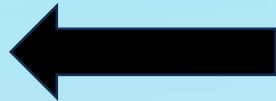
Leaf extract



Centrifuge



After centrifugation



Collect supernatant

2. NANOPARTICLE SYNTHESIS : SOL GEL METHOD



Zinc acetate



NaOH

Slowly add
Absolute ethanol



Ethanol is added to zinc acetate+NaOH mixture

MATERIALS REQUIRED

- Zinc acetate– 2 g in 15 ml
- NaOH -8 g in 10 ml
- Absolute ethanol



Zno nano-particles

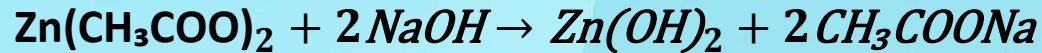


Filter



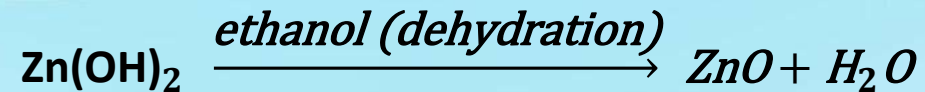
REACTIONS TAKING PLACE

1. When $\text{Zn}(\text{CH}_3\text{COO})_2$ (zinc acetate) reacts with NaOH , the immediate product is:

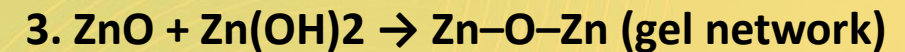
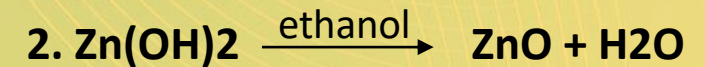
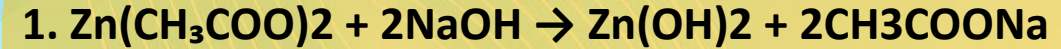


This forms **zinc hydroxide ($\text{Zn}(\text{OH})_2$)** — a gelatinous, unstable precipitate.

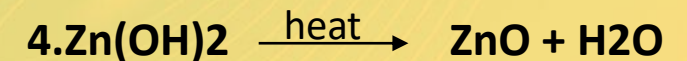
2. Absolute ethanol helps remove water:



Even at low temperature this reaction partially shifts toward **ZnO nuclei formation**.



CALCINATION



EXPERIMENTAL APPROACH

1. Assessment of Microbial Load on Food Packaging Materials After Storage.



Packaging covers taken

- Banana leaf
- Aluminium foil cover
- Plastic cover
- Butter paper

Food chosen

IDLI – (surface area is more and both side come in contact with the cover)

PROCEDURE



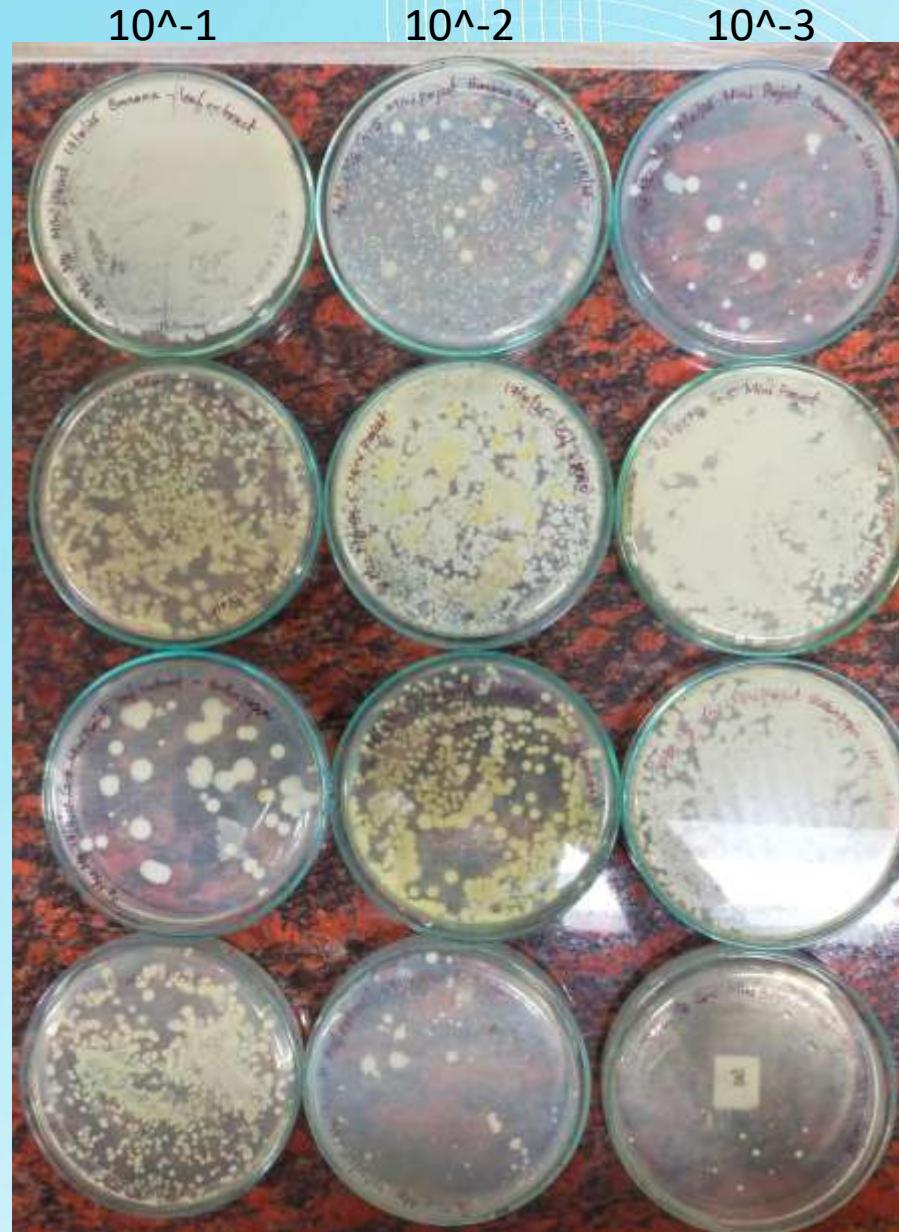
Idli is packed in different packing covers

Food is stored for 2 hrs



Serially diluted and plated (10^1 , 10^2 , 10^3 – dilutions)

Result



Plastic cover

Banana leaf

Butter paper

Aluminium foil

2.Preparation of Pure Microbial Cultures for Antimicrobial Assay of Plant Extracts



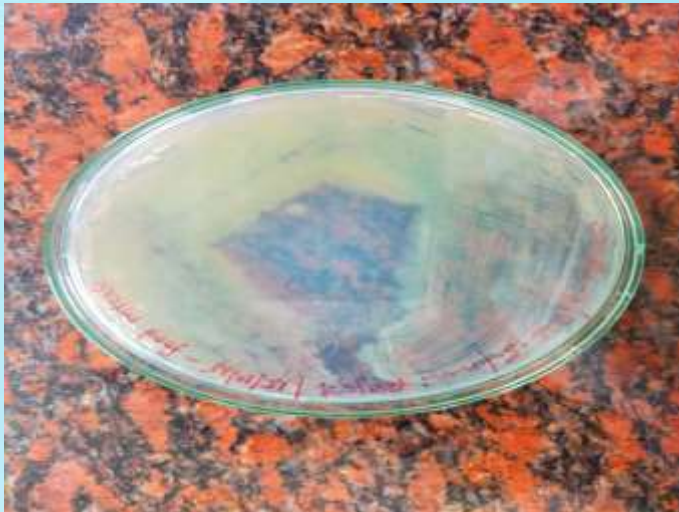
Pseudomonas sp.



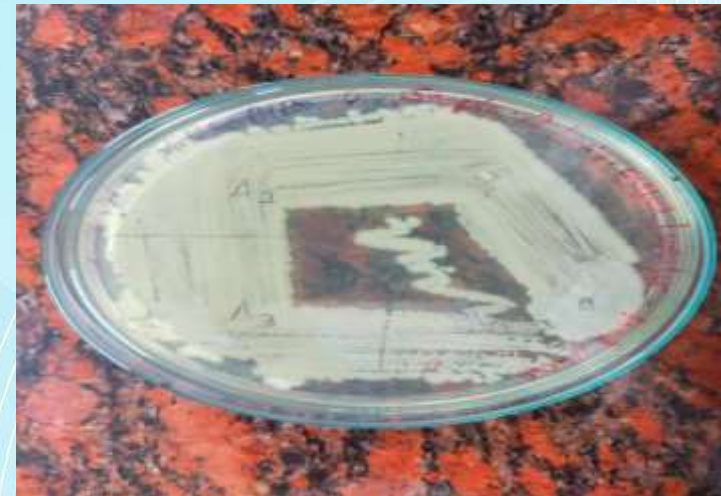
Staphylococcus sp.



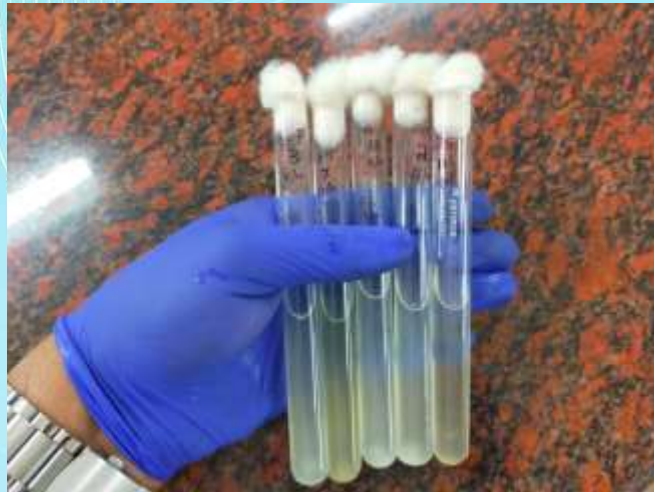
Klebsiella sp.



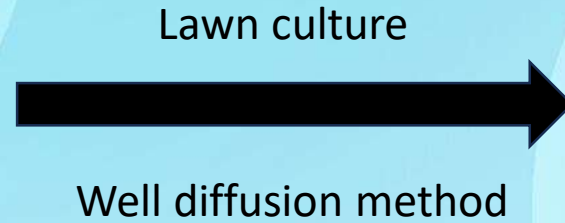
E.coli



Bacillus sp.



Bacterial cultures in broth



Antimicrobial activity testing

- 1.ZnO nanoparticle
- 2.Plant extract
- 3.Plant extract + ZnO
- 4.Control (D.W)

THE PLANT EXTRACT WAS USED WITHOUT BOILING OR HEATING (DIRECTLY TAKEN AFTER GRINDING)



Klebsiella sp.



E.coli



Pseudomonas sp.

- 1.ZnO NANOPARTICLE
- 2.Plant extract
- 3.Plant extract + ZnO
4. control



Bacillus sp.



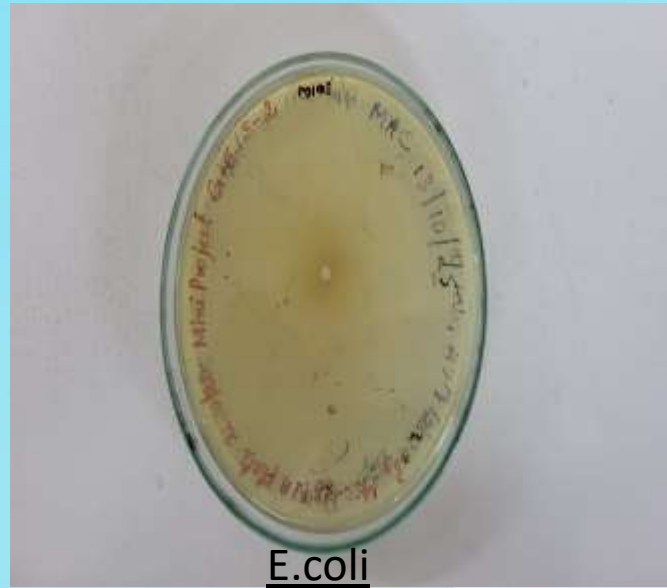
Staphylococcus sp.

RESULT : the ZnO nanoparticle showed no effect. The zone around plant extract showed contamination – may be presence Of Endophytic organism. Control showed no activity.

THE PLANT EXTRACT IS HEATED AT 100°C AND AGAIN PLATED



Klebsiella sp.



E.coli



Staphylococcus sp.



Pseudomonas sp.



Bacillus sp.

Result : after heating, the contamination was absent but there were no clear zone – May be because of the high dilution of the sample

3. Formulation Enhancement: Concentration and Chemical Augmentation of Plant Extract for Improved Antimicrobial Activity

Tween 20

enhances antimicrobial activity by increasing the permeability of microbial cell membranes.

Acetic Acid

Acetic acid exhibits antimicrobial activity by lowering the pH and penetrating microbial cell membranes in its undissociated form, where it disrupts intracellular enzyme systems and interferes with essential metabolic processes.

Hydrogen Peroxide (H₂O₂)

Hydrogen peroxide acts as a potent antimicrobial agent by generating reactive oxygen species (ROS) that oxidize cellular components, leading to membrane damage, protein denaturation, and DNA disruption in microbes.



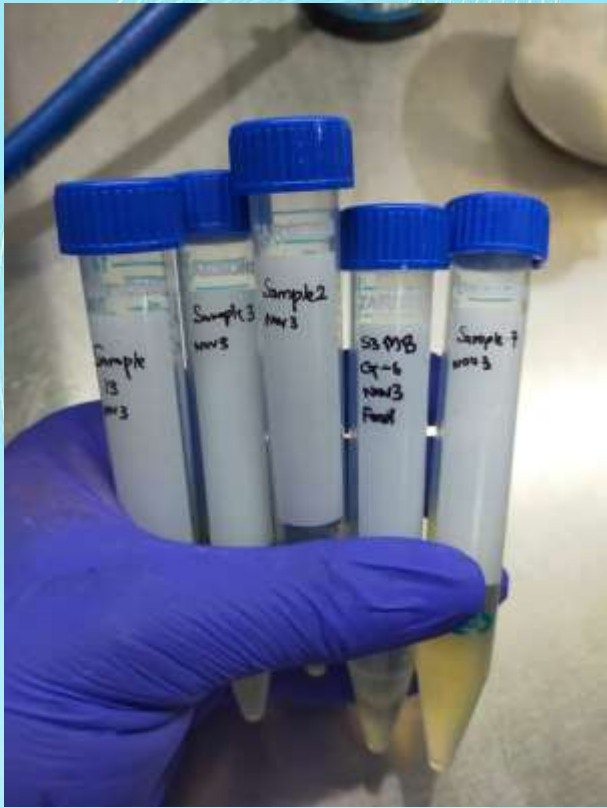
Tween 20



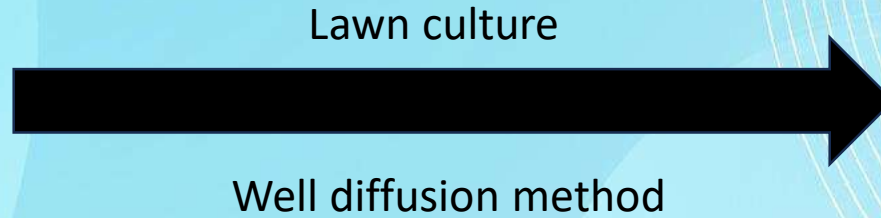
Acetic acid



H2O2



Bacterial pure culture



Antimicrobial activity testing

CONTROL

- H₂O₂
- Tween 20
- Acetic acid
- Crude plant extract

TEST

- Plant extract + H₂O₂
- Plant extract + tween 20
- Plant extract + acetic acid
- Plant extract + H₂O₂ + tween 20 + acetic acid



ZONE IN :
Acetic acid
H₂O₂
Tween 20

Staphylococcus sp.



ZONE IN :
Acetic acid
H₂O₂

E. coli



ZONE IN :
Acetic acid

Pseudomonas sp.

CONTROLS



ZONE IN :
Acetic acid
H₂O₂

Klebsiella sp.



ZONE IN :
Acetic acid
Tween 20

Bacillus sp.



E. coli



Staphylococcus sp.



Bacillus sp.

TEST SAMPLES

- Plant extract + H₂O₂
- Plant extract + tween 20
- Plant extract + acetic acid
- Plant extract + H₂O₂ +
tween 20 + acetic acid



Klebsiella sp.



Pseudomonas sp.

RESULTS

CONTROLS - Zone of inhibition were observed around acetic acid, tween 20, and H₂O₂ (concentrate).

Halo zones were observed around the Plant extract

TEST SAMPLES – HALO ZONES WERE OBSERVED AROUND :

- Plant extract + H₂O₂
- Plant extract + tween 20
- Plant extract + acetic acid
- Plant extract + H₂O₂ + tween 20 + acetic acid



4. Evaluation of Antimicrobial Effect of Concentrated Plant-Extract Coating: **Microbial Recovery from Food After 2-Hour Storage**



THE CRUDE PLANT EXTRACT (CONCENTRATED + HEAT TREATED) IS COATED ON THE SELECTED PACKING COVERS.

FOOD IS PACKED AND STORED FOR 2 HRS, THEN PLATED TO ANALYSE THE LOAD OF MICRO ORGANISMS.

RESULTS : THE LOAD OF MICRO ORGANISM IS REDUCED AND IN 10^2 & 10^3 DILUTIONS, THERE ARE MINIMUM COLONY.



BANANA LEAF



PLASTIC COVER



ALUMINIUM FOIL COVER

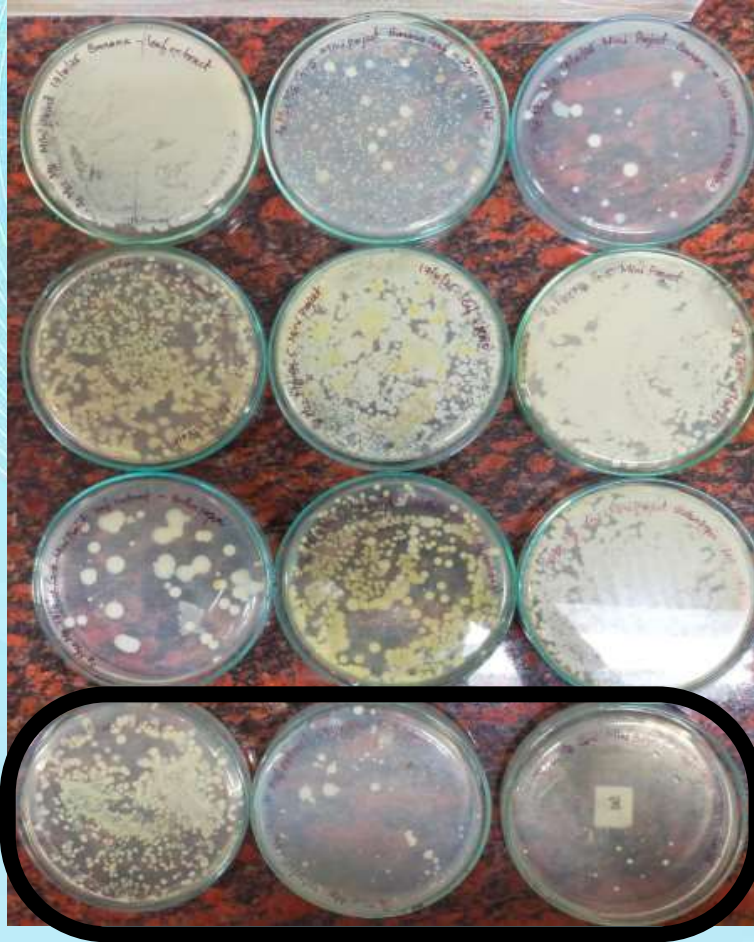


BUTTER PAPER



CONTROL

COMPARISON STUDY



REDUCTION IN MICROBIAL LOAD



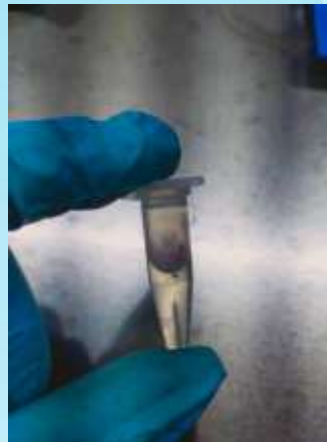
5. Development of an Antimicrobial Bio-Sheet Incorporating Concentrated Plant Extracts and Functional Additives, Followed by Microbial Evaluation



**Plant crude extract
(heated + concentrated)**



Tween 20



Acetic acid



H2O2

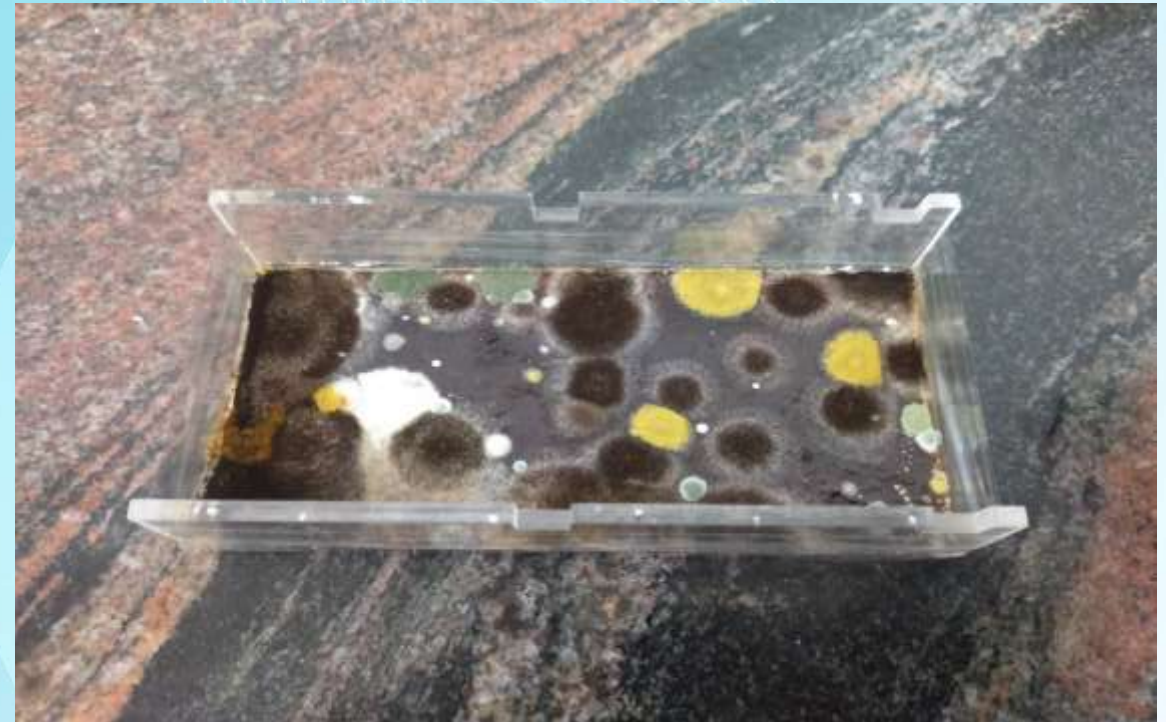


AGAROSE



GLYCERINE

RESULTS



THE GEL WAS CASTED ON A CASTING TRAY AND DRIED FOR 3 DAYS.

FUNGAL GROWTH CONTAMINATED THE PLATE BUT THERE WERE NO VISIBLE BACTERIAL COLONIES PRESENT IN THE GRIED GEL

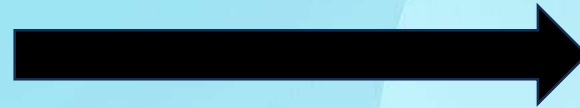
Incorporating Antifungal Agents to Prevent and Control Fungal Contamination in Packaged Products



The bio sheet after adding antifungal agent

- Amphotericin B
- Fluconazole

UV - treated



Food was packaged in the bio sheet, stored for 2 hrs,
And plated

RESULTS



THE FOOD PRODUCT (IDLI) WAS STORED IN THE BIO SHEET FOR 2 HOURS AND 10^1 DILUTION WAS PLATED IN NUTRIENT AGAR. THE PLATE SHOWED NO VISIBLE COLONIES.

THANK YOU....

**SPECIAL THANKS TO : MS. KALADEVI
AND ALL OTHERS WHO HELPED AND SUPPORTED US.**