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Antimicrobial activity of Neem Extract and Anti-Fungal activity of Turmeric Extract & making Emulsifying Cream

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Abstract **- An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called microbiostatic. The main classes of antimicrobial agents are disinfectants , which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth. In this paper we have preferred neem leaves against E. Coli and Bacillus subltilis and turmeric against Aspergillus niger.**

Index Terms **- Antimicrobial activity, Escherichia coli, Bacillus Subtilis, Aspergillus niger, Emulsifying Cream.**

INTRODUCTION

In 1928, Alexander Fleming became the first to discover a natural antimicrobial fungus known as Penicillium rubens. The substance extracted from the fungus he named penicillin and in 1942 it was successfully used to treat a Streptococcus infection. Penicillin also proved successful in the treatment of many other infectious diseases such as gonorrhea, strep throat and pneumonia, which were potentially fatal to patients up until then.
Many antimicrobial agents exist, for use against a wide range of infectious diseases.

Antibacterials :- Antibacterials are used to treat bacterial infections. The toxicity to humans and other animals from antibacterials is generally considered low. However, prolonged use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health. After prolonged antibacterial use consumption of probiotics and reasonable eating can help to replace destroyed gut flora.

Antifungals :- Antifungals are used to kill or prevent further growth of fungi. In medicine, they are used as a treatment for infections such as athlete's foot, ringworm and thrush and work by exploiting differences between mammalian and fungal cells. They kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the molecular level, making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side effects to some of these drugs. Some of these side effects can be life-threatening if the drug is not used properly
Antivirals :- Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host and therefore can be used to treat infections. They should be distinguished from viricides, which actively deactivate virus particles outside the body.

Antiparasitics :-Antiparasitics are a class of medications indicated for the treatment of infection by parasites, such as nematodes, cestodes, trematodes,

infectious protozoa, and amoebae. Like antifungals, they must kill the infecting pest without serious damage to the host.

Neem:-

Azadirachta indica is a tree in the mahogany family, Maliaceae. Neem is the most useful traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical and semi-tropical countries.

Its twigs are used as toothbrush and are widely used in the Indian sub-continent. Earlier studies on neem have showed that it contains active substances in almost every part of the seeds, leaves, roots, bark, trunk and branches with multiple medicinal properties.

Neem and its leaves used for the treatment of various diseases including eczema, ringworm, acne, anti-inflammatory activities, anti-hyperglycemic and also treat chronic wounds, diabetic foot and gangrene. It also removes toxins from the body; neutralize the free radicals present in body and used as blood purifier. Neem aqueous extract has powerful chemotherapeutic and viral agent. Recently it is reported as anticancer and used for hepatorenal protective activity and hypolipidemic effects(Kumar & Gupta, 2002)

To clean wounds, soothes, swellings and erases skin problems, boiled Neem is used. Neem leaves have been demonstrated to have vast properties like as, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic.

The anti-bacterial effects of crude extract of Neem seed against pathogens involved in eyes and ear infections.

I have performed the antimicrobial activity of Neem leaves against disease causing bacteria, such as Escherichia coli, Bacillus Subtilis

Turmeric:-

Curcuma longa (Turmeric) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to tropical South Asia and needs temperatures between 20 °C and 30°C and a considerable amount of annual rainfall to thrive.

It is a deep orange-yellow powder commonly used as a spice in curries and other South Asian and Middle Eastern cuisine, for dyeing, and to impart color to mustard condiments. The active ingredient in turmeric is curcumin, which has a distinctly earthy, slightly bitter, peppery flavor and a mustard smell. Turmeric has been used for over 2500 years in India, originally as a dye. It has become the key ingredient for many Indian, Persian and Thai dishes, not only in curry, but also in many more foods. Turmeric, which has a food additive code of E100, is also used to protect food products from sunlight.

The medicinal properties of this spice have been slowly revealing themselves over the centuries. Long known for its anti-inflammatory properties; turmeric has been deemed a natural wonder by recent research, proving beneficial in the treatment of many different health conditions from cancer to Alzheimer's disease. It is also a natural antiseptic and antibacterial agent, useful in disinfecting cuts and burns.

I have performed the antifungal activity of Turmeric against disease causing fungus, Aspergillus Niger.

Materials and Reagents:

Plant material: - Neem extract from neem plants leaves and Turmeric extract is collected from the market.

Bacterial cultures :-

1. Gram positive :- Bacillus subtilis
2. Gram negative :- Escherichia coli
3. Fungus :- Aspergillus Niger.

Chemicals:

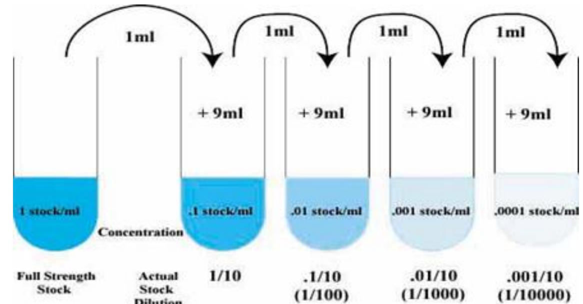
1. Ethanol as solvent for extraction of neem and turmeric extract.
2. Nutrient agar and nutrient broth for bacterial cultivation.

Equipment:

1. Autoclave
2. Laminar Air Flow
3. Incubator
4. Weighing machine (gram scale)

Isolation of gram-positive bacteria from soil:

Serial Dilution :



Requirements
- Test tubes
- Micropipette & Tips
- Distilled water
- Cotton plugs
- Marker
- Measuring Cylinder

Procedure
- 5 sterile test tubes were taken and labelled 10-1, 10-2, 10-3, 10-4 and 10-5.
- 1mL of soil sample was taken using a micropipette from the sample bottle and transferred aseptically to the test tube labelled 10-1.
- The contents were mixed thoroughly.
- 1mL of solution from 10-1 was then transferred to the test tube labelled 10-2 and these steps were repeated until 10-4 was transferred to 10-5.

Media Preparation
Requirements
- Paper
- Weighing machine (gram scale)
- Spatula
- 250mL conical flask
- Glass rod
- Cotton plug and String
- Autoclave
- Nutrient Agar Media

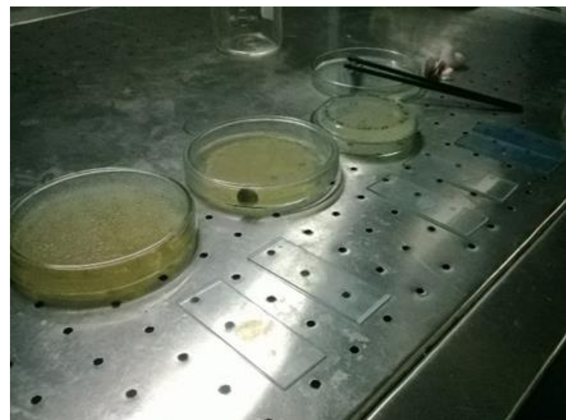
Media components
- Peptone - 1.25g
- Beef Extract - 0.75 g
- NaCl - 1.25g
- Agar - 5g
- Distilled Water - 250mL

Procedure
- All the components were measured in exact proportions and added to the conical flask.
- The volume was made up to 250mL by adding distilled water.
- The components were homogenized using a glass rod.

The flask was plugged properly and autoclaved to make the media sterile

Inoculation and Incubation

Requirements
- Laminar airflow
- Sterile petri-plates
- Spreader
- Alcohol 70%
- Micropipette and Tips
- Marker
- Serial Dilution test-tubes
- Incubator

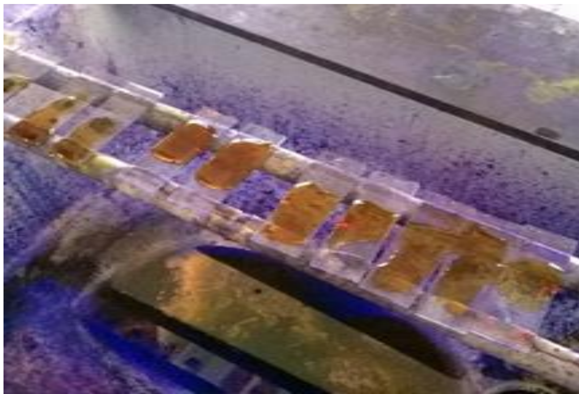


Procedure
- All the steps were carried out under a laminar airflow.
- The petri plates were labelled 10-1, 10-2up to 10-5 on each.
- The NA media was poured onto each plate ~20mL each and allowed to solidify.
- 100µl of sample from each dilution was added dropwise to each of its respective petri-plate.

- Using a spreader dipped in alcohol and flamed, the sample which was added previously was spread all-over the media surface.
- The lids were flamed and covered.
- All the 5 plates were kept in an incubator for 24-48hrs at 37ºC.
- The results were seen and plotted accordingly.

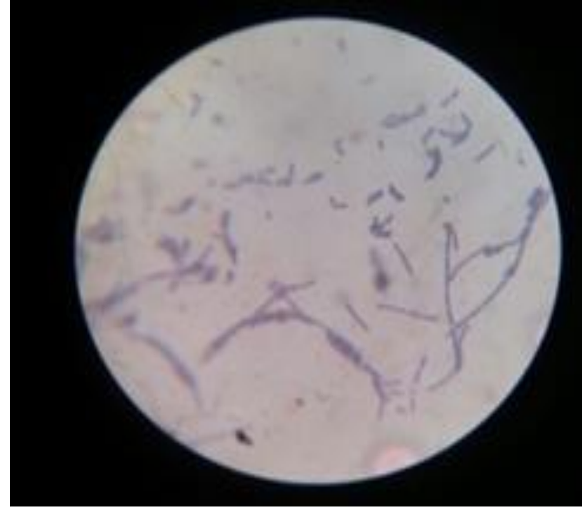
Staining and Visualization

Requirements
- Inoculation loop
- Spirit lamp
- Bacterial Culture
- Crystal violet
- Distilled water
- Ethanol 70%
- Safranin
- Iodine solution
- Glass Slides
- Seeder Oil
- Microscope with 100X objective lens



Procedure
- A clean and dry glass slide was taken and a smear was made on it by taking a loop-full of bacterial culture from a marked area from culture plate 10-5.
- It was air- dried and heat fixed.
- The slide was stained first using Crystal violet stain.
- It was washed with distilled water and then Iodine was added.
- Ethanol 70% was poured and then washed with distilled water.
- Finally counter-stain safranin was added and the slides were washed and dried.
- The slides were seen under a 100X lens using a drop of oil.

Observation

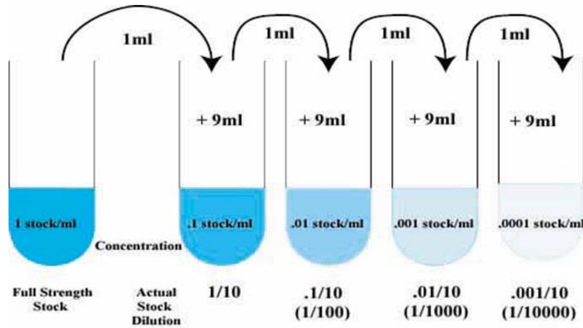


Microscopic view of bacillus after staining

Isolation of gram-negative bacteria from drainage water

Serial Dilution :
Requirements
- Test tubes
- Micropipette & Tips
- Distilled water
- Cotton plugs
- Marker
- Measuring Cylinder

Procedure



- 5 sterile test tubes were taken and labelled 10-1, 10-2, 10-3, 10-4 and 10-5.
- 1mL of drainage water sample was taken using a micropipette from the sample bottle and transferred aseptically to the test tube labelled 10-1.
- The contents were mixed thoroughly.
- 1mL of solution from 10-1 was then transferred to the test tube labelled 10-2 and these steps were repeated until 10-4 was transferred to 10-5.

Media Preparation

Requirements
- Paper
- Weighing machine (gram scale)
- Spatula
- 250mL conical flask
- Glass rod
- Cotton plug and String
- Autoclave
- Nutrient Agar Media

Media components
- Peptone - 1.25g
- Beef Extract - 0.75 g
- NaCl - 1.25g
- Agar - 5g
- Distilled Water - 250mL

Procedure
- 50mL distilled water was added to the conical flask after measuring.
- All the components were measured in exact proportions and added to the conical flask.
- The volume was made up to 100mL by adding distilled water.
- The components were homogenized using a glass rod.

The flask was plugged properly and autoclaved to make the media sterile

Inoculation and Incubation

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- Micropipette and Tips
- Marker
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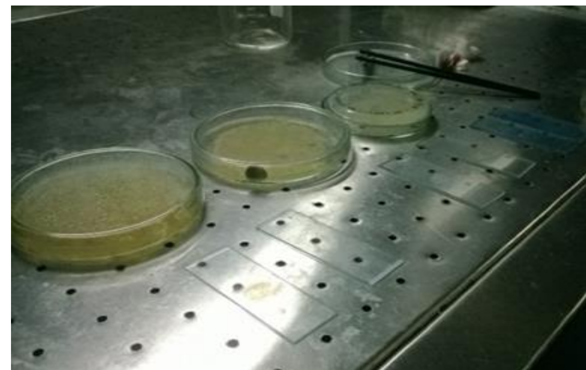

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- The petri plates were labelled 10-1, 10-2up to 10-5 on each.
- The NA media was poured onto each plate ~20mL each and allowed to solidify.

- 100µl of sample from each dilution was added drop-wise to each of its respective petri-plate.
- Using a spreader dipped in alcohol and flamed, the sample which was added previously was spread all-over the media surface.
- The lids were flamed and covered.
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Staining and Visualization

Requirements

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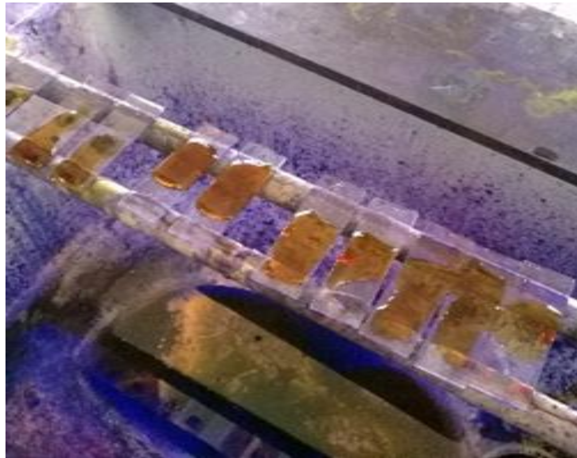

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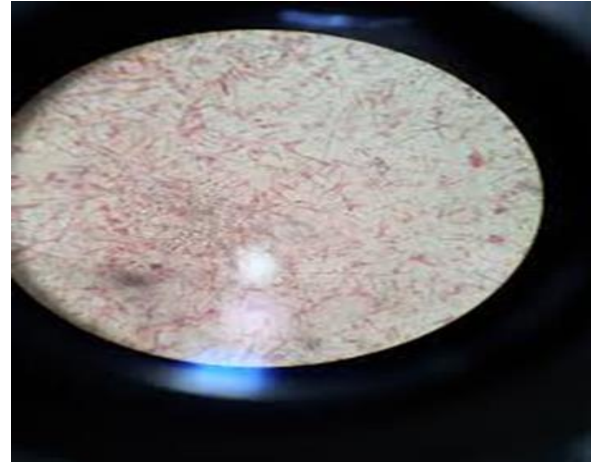
- The slides were seen under a 100X lens using a drop of oil.

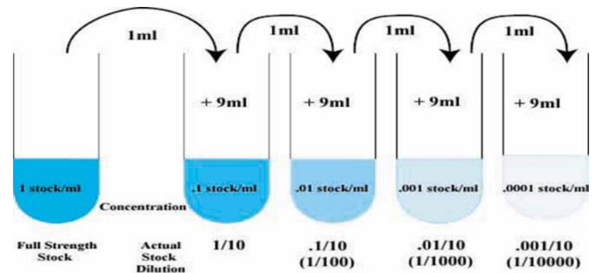
Observation:



Microscopic view of E.coli after staining

Isolation of Fungus from bread:-

Serial Dilution :



Requirements
- Test tubes
- Micropipette & Tips
- Distilled water
- Cotton plugs
- Marker
- Measuring Cylinder

Procedure
- 5 sterile test tubes were taken and labelled 10-1, 10-2, 10-3, 10-4 and 10-5.
- 1mL of drainage water sample was taken using a micropipette from the sample bottle and transferred aseptically to the test tube labelled 10-1.
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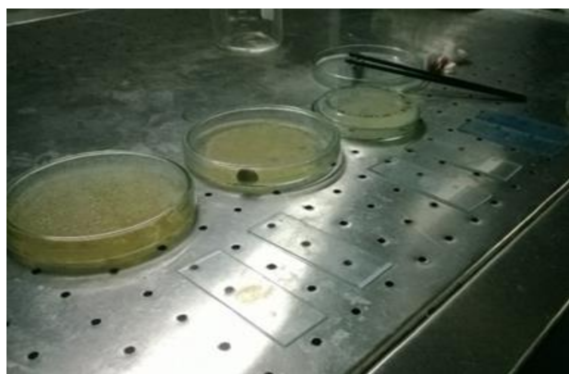

Requirements
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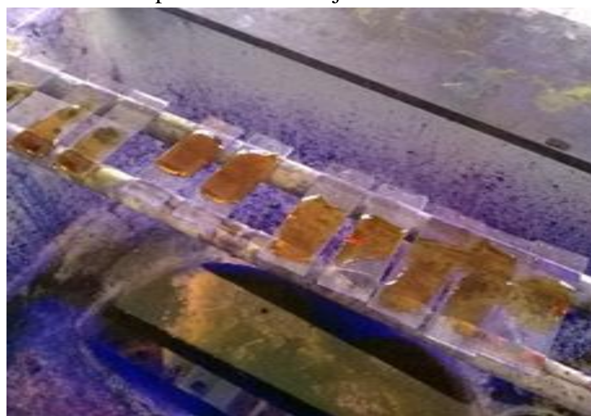

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- Ethanol 70% was poured and then washed with distilled water.
- Finally counter-stain safranin was added and the slides were washed and dried.
- The slides were seen under a 100X lens using a drop of oil.

Observation:
Microscopic view of Aspergillus Niger after staining

Preparation of the extract
Neem
- Neem leaf is collected from the neem plant.
-
- The leaves are then wrapped with newspaper & dried 7-10 days.
- After that the dried leaves are mixed in a mixer grinder to get powder of the leaves.
-
- The neem powder is then transferred to a beaker with 100ml ethanol for maceration 20-25 days.

- Next the macerated neem powder is collected in a beaker & wrapped a foil over it. The foil should have pores for the evaporation of the ethanol.
- After two days extract is found.
- Weight of the extract is-4.03 gm.

Turmeric

- Raw Turmeric is being collected from the local market.

- Cut into small pieces & wrapped in newspaper and dried 7-10 days.

- After drying it is transferred to a beaker with 100ml ethanol for maceration 20-25 days.

- The next procedure is as same as neem .
- The extract's weight is-1.629gm

The extracts are-

Antimicrobial activity test of the neem extract:

MINIMAL INHIBITORY CONCENTRATION (MIC)

Principle: In microbiology, minimum inhibitory concentration is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against organism.

Material's Required

- Biological sample:-E.coli, Bacillus Subtilis, Aspergillus Niger.

- Chemicals:-Nutrient agar media, Erythromycin antibiotic, alcohol (70%)
- Glass wares/lab wares:-Petriplates for ceps, filter paper disc, spreader, burner, micropipette,
- Instruments:- Laminar air flow, incubator, weighing balance, pH meter, Autoclave.

For NEEM extract

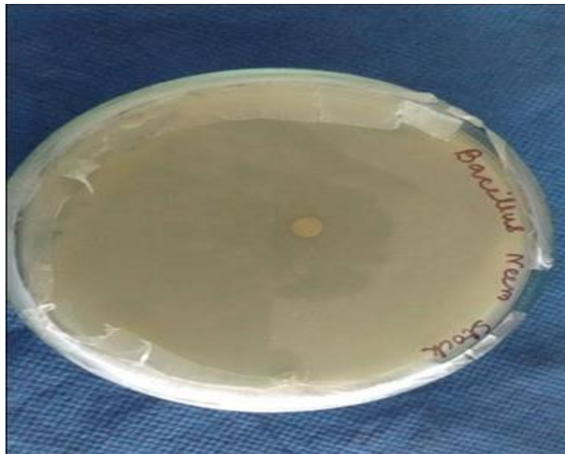
Procedure :-

- 6 nutrient agar plates were prepared and autoclaved allowed to solidify.
- 2 above mentioned test organism were selected.
- The test organism of bacillus was inoculated in the 3 Petri plates of media as a lawn culture.
- The test organism of E.coli was inoculated in the another 3 Petri plates of media as a lawn culture
- Then, the plates were allowed to dry for 5 minutes.
- With the help of a forceps sterile paper discs were dissolved in different dilution (Stock,10 -1 , 10 -2 , 10 -3, 10 -4) of extract.
- Each Petri plate is divided into two quadrants.
- Then, two discs were placed ascetically in each quadrant of the plates.
- The plates were then incubated for 24 hrs at 37 degree centigrade.
- The zone of inhibition was then measured by a ruler in each of the plate.

Result

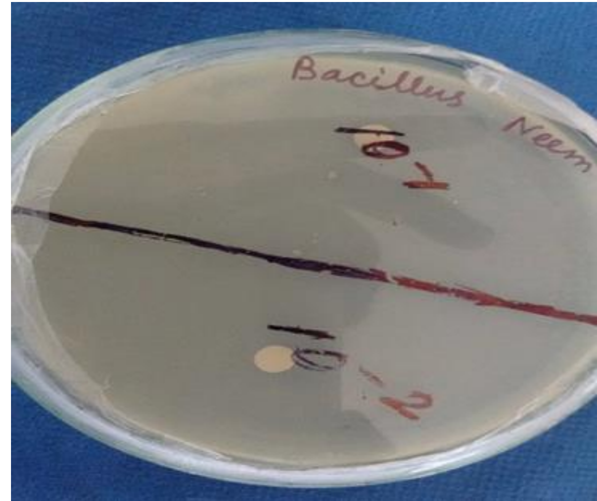
The zone of inhibition was measured for Neem extract and the result depicted bellow:-

Results for gram positive bacteria



Bacillus stock:- 0.8 cm

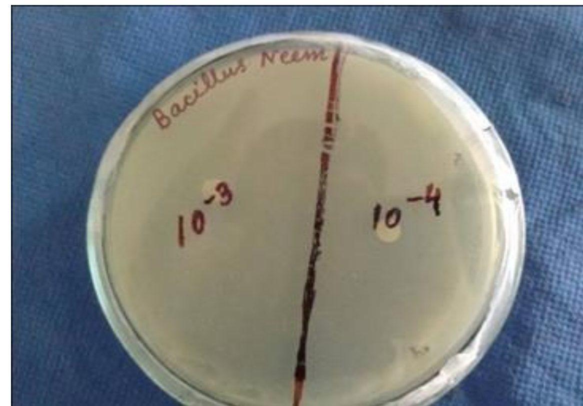
Bacillus 10^{-1}:-0.8cm



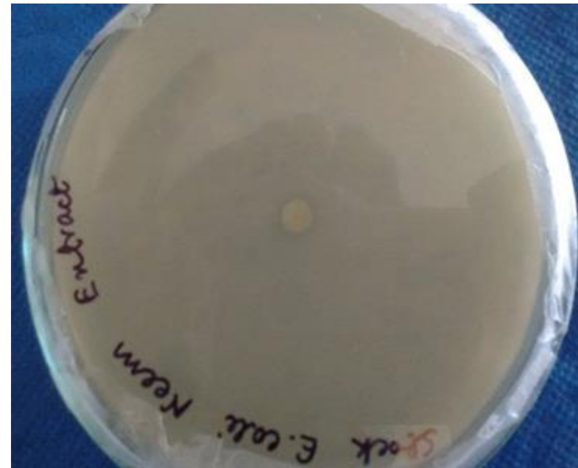
Bacillus 10^{-1}:- 0.8cm

Bacillus 10^{-3}:-0.65cm

Bacillus 10^{-4}:-0.8c

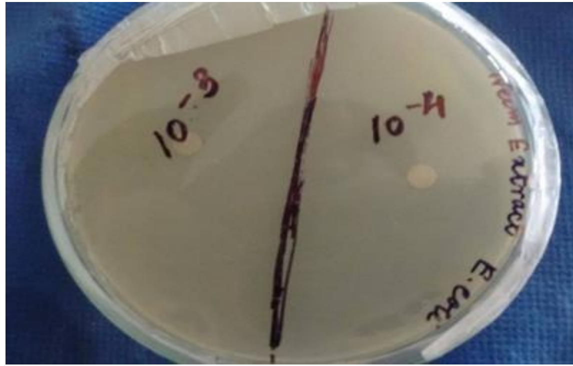


Result for gram negative bacteria



E.Coli Stock:-0.80 cm

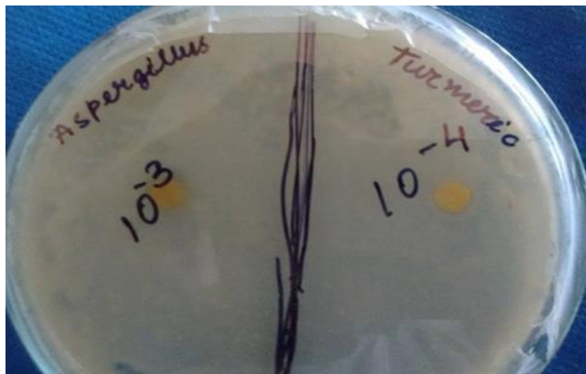
E.Coli 10^{-3}:-0.75 cm   E.coli 10^{-4}:-0.85 cm



Result for fungal activity Turmeric in aspergillus Niger:-

Aspergillus Niger Stock:- 0.75cm

Aspergillus Niger 10^{-3}:0.60 Aspergillus Niger 10^{-4} :0.75cm

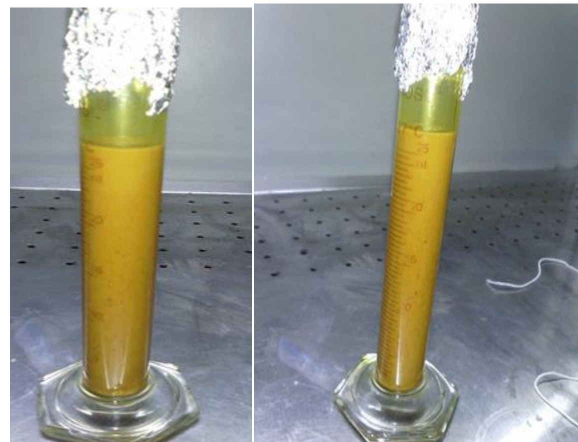


Procedure of making emulsifying cream

Requirements :-
Light liquid Paraffin
Span 80
Distilled Water
Tween 80
Neem Extract
Turmeric Extract
Beaker
Glass rod
Stirrer / Homogenizer

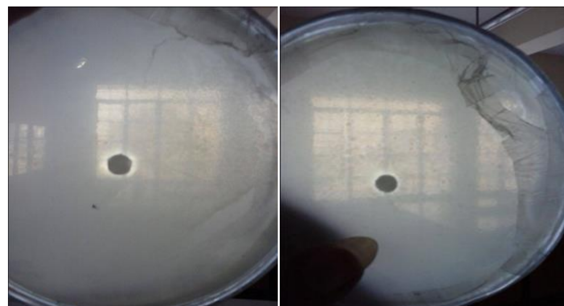
Procedure:-
Taken 4ml of the Light liquid Paraffin and mixed 1ml Span 80 with it in a beaker and continuously stir it with the help of a glass rod.
Leave this for 5-10 mins.
With the help of measuring cylinder 16ml distilled water is measured & 2ml Tween 80, 1ml Neem extract stock solution & 1 ml of Turmeric extract stock is mixed with this.
Leave it for 5 mins with continuous shaking
After that the mixture of the Span 80 & light liquid paraffin is mixed slowly (drop by drop) when the other solution is stirred in the homogenizer machine.
Homogenize 1 hour and the emulsifying cream is ready.

This is the emulsifying cream:-



MIC of the emulsifying cream

Result:-



Zone of inhibition:- 0.90 cm

ACKNOWLEDGMENT

I would like to special thanks of gratitude of my University Department, my colleagues and Abu. Syed Md, Sonia Bajaj, B. P. Srinivasan, R. N. Chopra for their previous work where I took concept of this work.

CONCLUSION

The crude extract of neem displayed excellent growth of inhibition on gram positive bacteria Bacillus. The MIC values of gram-positive bacteria are 0.8cm, 0.7cm,0.65cm, 0.80cm the gram-negative bacteria E.coli displayed the growth of inhibition. But it shows the growth of inhibition only for two dilutions of 10 -3, 10 -4. The MIC values of gram-negative bacteria are 0.75cm, 0.85cm.
Both stock extraction MIC is 0.80cm
The crude Extract of Turmeric displayed the growth of inhibition against Aspergillus Niger for two dilutions 10-3, 10-4. The MIC values are 0.60cm & 0.75 cm
MIC of the stock solution is 0.75cm
After mixing the two extracts, the MIC result of the Emulsifying Cream is – 0.90cm

REFERRENCES

[1] R. N. Chopra, S. L. Nayer, and I. C. Chopra, I.C.- Glossary of Indian Medicinal plant, The University of Chicago Press Journals,vol 33(2), pp. 156.1958.
[2] A. S. Md, M. M. Md, U. Rashid - A comparative study of Anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone. Bangladesh J of Pharmacol, vol.3, pp.44-47. 2008.
[3] K. Almas, T. R. Ansallafi,- The natural toothbrush. World health Forum. vol. 16, pp. 206-210. 1995.
[4] B. Sonia, B. P. Srinivasan, B.P.-Investigation into the Anti-diabetic activity of Azadirachta indica. Indian journal of pharmacology vol 31, pp. 138-141. 1999.
[5] A. K. Saseed, and A. Junaid- Study on the effect of neem (Azadirachta indica A. juss) leaf extract on the growth of Aspergillus parasiticus and production of aflatoxin by it at different incubation times. Journal compilation, vol. 51, pp. 35-39. 2008.
[6] B. Kausik, C. Ishita, K. B. Ranajit, B. Uday- Biological activities and medicinal properties of neem (Azadirachta indica). Current Science, vol. 82(11), pp. 1336-1345.2002.
[7] B. H. Ali, N. AL Wabel, G. Blunden- Phytochamical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.A. review. Phytotherapy Reasearch, vol. 19(5), pp. 369-375. 2005.
[8] R. Jepson, J. Craig, Cranberris for preventing urinary tract infection- The Cochrane Collaboration, vol. 10, pp. 1-80. 2008.