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Original Article

Anti-Microbial Activity of Antibiotic Produced From Fungi by Fermentation Process.

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Abstract

Antibiotics are among the most frequently prescribed medications in modern medicine. Antibiotics cure disease by killing bacteria and keeping them from reproducing. Penicillin was used as standard antibiotics to evaluate the anti-microbial activity. In vitro fungal extract (*Aspergillus niger and Penicillium notatum*) are isolated and tested against micro-organism (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis*) for detection of its anti-microbial activity. This test is performed by using cup plate method and observes its activity by measuring zone of inhibition. Minimum inhibitory concentration is used for the detection of minimum conc. of extract used to inhibit microbial growth.

Keywords: Fungal extract, Cup plate method, MIC, Bacteria, Zone of Inhibition.

1. Introduction

Penicillin is a group of antibiotics derived from Penicillium fungi. Penicillin is a historically significant drug being the first antibiotic discovered. Penicillin is a Beta lactam antibiotic and is used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms[1]. In September 1928, Alexander Fleming accidentally discovered penicillin when he was engaged in studying different kinds of bacteria and molds in his laboratory. He once opened one of his petri dishes for a few seconds to smear it with a strain of staphylococcus which is a bacterium that typically occurs in clusters resembling grapes. Fleming noticed a halo of inhibition of bacterial growth around a contaminant blue-green mould staphylococcus plate culture. He concluded that the mould was releasing a substance that was inhibiting bacterial growth and lysing the bacteria. He grew a pure culture of the mould and discovered that it was a penicillium mould, now known to be penicillium notatum. Fleming carefully preserved the culture, but the discovery lay essentially dormant for over a decade[2].

Penicillin was the first naturally occurring antibiotic discovered. It is obtained in a number of forms from Penicillium moulds. Penicillin is not a single compound but a group of closely related compounds, all with the same basic ring-like structure (a β-lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate[3]. The third amino acid of this tripeptide is replaced by an acyl group (R) and the nature of this acyl group produces specific properties on different types of penicillin. Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For

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example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics[4].

2. Materials and Methods

2.1. Requirement

1) Chemicals: beef extract, peptone, glucose, sodium chloride, agar etc.
2) Apparatus: conical flask, glass rod, burner, Petri plate, test tube, measuring cylinder, cotton etc.
3) Equipment: autoclave, oven, PH meter, balance.
4) Microbial Culture-*S.aureus, B.substilis, E.coli, Aspergillus niger, P.notatum.*

Procedure for the Isolation of Antibiotic from fungi *Aspergillus Niger*

After 6-8 days of batch culture, the liquid medium is pumped out, filtered and concentrated. The basic antibiotic - benzyl penicillin - is precipitated as crystals when potassium compounds are added. This antibiotic may then be modified by the action of other micro-organisms or by chemical means, before being mixed with inert substances and pressed into tablets or converted into syrup or inject able form[5]. Although the molecular structure of penicillin is known, and it may be synthesized by chemical methods, it is not economic to do so. The production process still relies on fungal fermentation based on biological principles, although modern strains are much more productive than the early strains. This has been achieved through screening programmes involving isolates from different sources, and treatment to encourage mutations[6].

Microbial assay

Many therapeutic agents, which either inhibit the growth of microorganism (antibiotic) can be standardized by microbiological assays. Any change in the antibiotic molecule which may not be detected by chemical methods will be revealed by a change in the antimicrobial activity and hence microbiological assays are very useful for resolving doubts regarding possible change in the potency of antibiotics. The minimal assay is based upon a comparison of the inhibition of growth of microorganisms by measured concentrations of the antibiotics to be examined with that produced by known concentration of standard preparation of the antibiotic having a known activity. Two general methods are used for microbiological assays of antibiotics[7].

Determination of zone of inhibition by cup plate method

The antibacterial activity of acetonolic extract was performed using agar cup plate method. 20ml of sterile nutrient agar medium was poured into sterile petriplates and allow solidifying. The antibacterial activity of acetonolic extract was performed using agar cup plate method. 20ml of sterile nutrient agar medium was poured into sterile petriplates and allow solidifying. The petriplates were incubated at 37ºc for 24 hours to check for sterility the medium was seeded with the organisms by pour plate methods using sterile top agar (4ml) contained 1ml culture. The wells are prepared in a media by sterile cork borer. And add sample in increasing conc. In a respective well. The plates were incubated at 4ºc for ½ hours for diffusion of test extract. After diffusion, the Petri dishes were incubated at 37ºc for 24 hours and zone of inhibition were observed and measured[8].

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration is the minimum concentration of antimicrobial compound found to inhibit growth of particular test microorganism. It is apply to disinfectant, antiseptic, preservative and antibiotics. MIC of different antimicrobials is determined by broth/liquid dilution method or solid dilution method [9, 10].

Broth dilution method

In this method, liquid nutrient broth media were prepared, poured into test tube to this antimicrobial compound and sterile water was added. It is given in the table no.1.1

Results and Discussion

Determination of Zone of inhibition by cup plate: method

The sample shows the zone of inhibition by cup plate method against the B. Subtilis, and E. coli bacteria. Observe all the plates for the zone of inhibition and record the diameter in the observation table. Observe the zone of inhibition around the cavity[11, 12]. Measure the

diameter by zone reader and record in the observation table.

Minimum inhibitory concentration

After incubation, all test tubes or Petri plates are examined for the growth in form of turbidity and colonies, respectively. Record the results and calculate minimum inhibitory concentration by comparing all results with positive and negative control[13].

Test tube no.	Concentration	Optical density
Blank (water)	-	00
Control	-	01
1	1mg	0.56
2	2mg	0.53
3	3mg	0.51
4	4mg	0.48

Conclusion

The work undertaken was to determine the antimicrobial activity of extract of Aspergillus niger. The crystal of Aspergillus niger was extracted by using charcoal to remove the impurity and potassium hydroxide to form the crystal. This crystal was diluted with sterile water and use for the antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, E.coli, and A. Niger as a standard drug by cup plate method. The present work concluded that crystal extract of penicillium notatum showed antimicrobial activity but somewhat less than that of the standard drug, this result can be used for further studies on antimicrobial activity of penicillium notatum.

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Fig 1. Zone of inhibition of *E.coli*

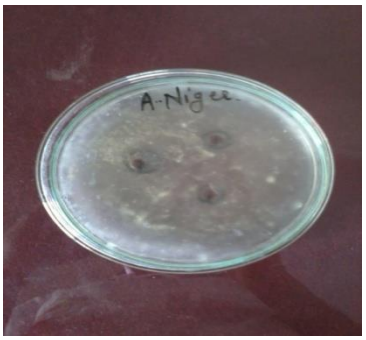

Fig 2. Zone of inhibition of *A. Niger*

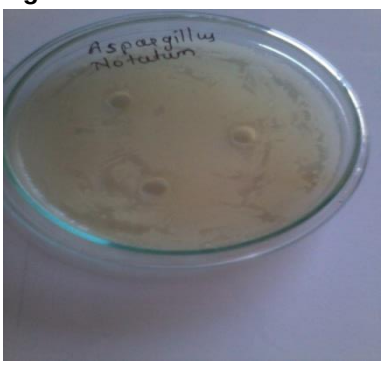

Fig 3. Zone of inhibition of *A. Notatum*

Fig 4. Zone of inhibition of *S. aureus*

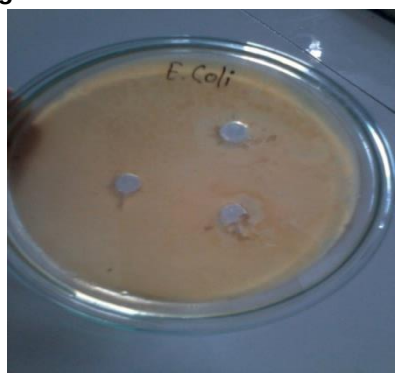

Fig 5. Zone of inhibition of *E. coli*

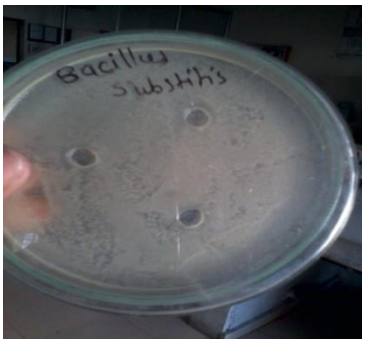

Fig 6. Zone of inhibition of *B. subtilis*

Table 1. Standardization by Broth dilution method.

Test tube no.	Volume of double strength medium (ml)	Volume of test antimicrobial compound (ml)	Volume of sterile water (ml)
Blank	5	-	-
Control	5	-	-
1	5	1	5
2	5	2	4
3	5	3	3
4	5	4	2

Table 2. Evaluation of antimicrobial activity of fungal extract using cup plate method.

Sr. No.	Organism	Concentration of sample in mg/ml and zone of inhibition in cm				
		1mg	2mg	3mg	4mg	5mg
1	***E. coli***	0.7	0.7	0.6	0.6	0.8
2	***B. subtilis***	0.4	0.5	0.4	0.6	0.4
3	***S. aureus***	0.4	0.6	0.5	0.5	0.6
4	***A. niger***	0.3	0.4	0.5	0.5	0.4

Table 3. Evaluation of antimicrobial activity of standard antibiotic using cup plate method.

Sr. No.	Standard drug	Concentration of sample in mg/ml and zone of inhibition in cm				
1	Penicillin	1mg	2mg	3mg	4mg	5mg
		0.8	1	0.8	1.1	1.3

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