



Comparative Analysis of Penicillin Production Using Various Media Compositions: A Research Study

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ABSTRACT

Background: Penicillin are bactericidal beta-lactam antibiotics that inhibit bacterial cell wall synthesis. A natural product, the penicillin structure has been modified to prepare a variety of semi-synthetic agents and used in pharmaceutical industries. The spectrum of antibacterial activity varies with each class of the penicillin family produced by penicillium species. Penicillin is generally well-tolerated, with hypersensitivity being the major adverse effect. Penicillin is used to treat a variety of conditions including skin infections, urinary tract infections, upper and lower respiratory infections, and endocarditis.

Methods: Preparation of different media compositions to check the increased growth of *Penicillium notatum* and its ability to produce penicillin crystals using the varied compositions. The obtained penicillin from the extract we checked for the antibacterial activity against various microbial species.

Result: We have successfully established that Penicillin can be useful drug for the treatment of serious *Pseudomonas aeruginosa* infections because of its enhanced activity against this organism. This indicates that Penicillin still could serve as medicinal drug and antimicrobial activity in pharmaceuticals for treatment of many pathogenic diseases.

Conclusions: The above research work concludes that high yield of penicillin production can be obtained using sabouraud dextrose broth medium with wheat bran as the substrate in industrial means. It shows the efficacy of the Penicillin against various infection and can inhibit the growth of some organisms causing infection effectively.

Keywords: Substrate wheat bran, Sabouraud Dextrose Broth, Rose Bengal Broth, Potato dextrose Broth, *Penicillium*, bacterial test organisms.

INTRODUCTION

Penicillin is one of the most important and widely used antibiotics in modern medicine. It is a group of naturally occurring and synthetic antibiotics that are effective against a broad spectrum of bacterial infections. The discovery of penicillin marked a significant milestone in the history of medicine and revolutionized the treatment of infectious diseases. Penicillin was discovered by Sir Alexander Fleming, a Scottish bacteriologist, in 1928 (BornsidesGH, 1968). While working at St. Mary's Hospital in London, he accidentally observed that a mold called *Penicillium notatum* had contaminated a bacterial culture plate, leading to the inhibition of bacterial growth around the mold. He identified this mold as the source of a potent antibacterial substance, which he named penicillin (Bodey G P,1971). Penicillin is an antibiotic, which means it is a type of medication that kills or inhibits the growth of bacteria. It targets the bacterial cell wall, disrupting its formation and weakening the bacteria, ultimately leading to bacterial cell death.

After Fleming's initial discovery, further research was conducted to isolate and produce penicillin in larger quantities. This involved the work of scientists Howard Florey, Ernst Boris Chain, and Norman Heatley at the University of Oxford during the 1940s (Schatz *et.al.*, 1944). They successfully developed methods to mass-produce penicillin, which played a crucial role in treating wounded soldiers during World War II. Penicillin and its derivatives have saved countless lives since their introduction into medical practice. Prior to its discovery, bacterial infections were a leading cause of death, and treatment options were limited. Penicillin was a breakthrough in the fight against bacterial diseases like pneumonia, strep throat, syphilis, and various skin infections (Landesman *et.al.*, 1981). Over time, various forms of penicillin have been developed to enhance their effectiveness against different types of bacteria and to address issues of resistance. The substrate used for penicillin production is typically a nutrient-rich medium that provides essential nutrients for the growth of the *Penicillium* mold, which is the microorganism responsible for producing penicillin. *Penicillium* fungi require a carbon source, nitrogen source, minerals, and other growth factors to thrive and produce penicillin (Rocha *et. al.*,2019). The primary substrate used for industrial-scale penicillin production is a liquid medium, often based on corn steep liquor or lactose as the carbon source and a nitrogen source such as corn steep liquor, soybean meal, or a combination of both. The medium may also contain various salts, vitamins, and other growth-promoting agents. The process of penicillin

production involves fermentation, during which the *Penicillium* mold is grown in large fermentation tanks or bioreactors using the nutrient-rich substrate. The mold secretes penicillin into the medium during its growth phase, and once the desired level of penicillin production is achieved, the fermentation is halted, and the penicillin is isolated and purified from the medium for further processing and use as an antibiotic. Penicillin is a highly effective antibiotic that exhibits potent antibacterial activity against a wide range of bacteria. It is particularly effective against Gram-positive bacteria. The antibacterial activity of penicillin is primarily due to its ability to interfere with bacterial cell wall synthesis (Dede *et al.*, 2020).

Around the world, antibiotics are essential for controlling infectious ailments that affect people, animals, cattle, and aquacultures. The potential hazard to all microorganisms in these habitats is created by the introduction of an increasing number of antibiotics into soils and streams (Vazquez-Munoz *et al.*, 2019). It is crucial to create innovative antibiotic agents and therapies to control bacterial infections because the capacity of microorganisms to build resistance out competes the production of new and effective antibiotics (Cycon M, 2019).

In this study we have experimented with numerous media compositions in search of the simplest means to produce penicillin, checking the increasing production as we want. Penicillin primarily disrupts the production of the bacterial cell wall, which leads to an incorrect construction of the cell wall. This ultimately causes the cell to rupture by allowing water to enter the cell wall. The zone development surrounding the well with penicillin on a culture plate can be used to pinpoint the location (Girolami *et al.*, 1980).

METHOD

1. Isolation of *Penicillium*

10 gm of garden soil sample was collected and serial dilution was performed. Spread plate method was conducted in the Sabouraud Dextrose agar medium. The plates were incubated at room temperature for 7 days (Schwartzkroin *et al.*, 1977).

2. Staining Technique

After the incubation period the organism was stained using Lactophenol cotton blue stain.

3. Production Media Preparation

Three different mediums (Sabouraud Dextrose Broth, Rose Bengal Broth, Potato dextrose Broth) were prepared for 200ml and added 1g of wheat bran to each medium as substrate. The production media was sterilised in an autoclave at 121°C for 15 mins. After sterilisation, cool down the production media to room temperature. The isolated *Penicillium sp.* was inoculated in the three different production media and kept for incubation for 25 days at room temperature.

4. Extraction of penicillin

The mat is taken out and crushed with the help of mortar and pestle using 50 ml of sterile distilled water. The remaining broth was filtered using Whatman no.1 filter paper. The crushed mat and the broth were centrifuged at 8000 rpm for 15 mins. The supernatant was collected in a separate tube and tested for antimicrobial activity (Sharifzadeh *et al.*, 2020).

5. Antimicrobial test

9 Muller Hinton agar plates were prepared and sterilised in an autoclave at 121°C for 15 minutes. After the sterilisation the media were allowed to cool and poured into the respective sterile Petri plates and kept for solidification. After solidification of media four wells were cut by aseptic method in each plate (Lopez *et al.*, 2001). Using a sterile cotton swab 9 different organisms like *Salmonella sp.*, *Vibrio sp.*, *Staphylococcus aureus*, *Staphylococcus cohnii*, *Rhodococcus sp.*, *Klebsiella sp.*, *Pseudomonas sp.*, *Candida sp.*, *Bacillus sp.* were swabbed on each plate. Different concentrations (0.1, 0.3, 0.5ml) of the filtrate was added to the well and one well acted as control without adding the filtrate. The plates were incubated for 24 hours at 37°C.

RESULT

The findings of this study indicate that Penicillin has potential value in the management of many types of serious community-acquired infections. The organisms that cause these infections, with the exception of *Staphylococcus aureus*, are likely to be susceptible to Penicillin. Penicillin may also be a useful drug for the treatment of serious *Pseudomonas aeruginosa* infections because of its enhanced activity against this organism. These results indicate that Penicillin still could serve as medicinal drug and antimicrobial activity in pharmaceuticals for treatment of many pathogenic diseases.



Fig-1: Growth of *Penicillium notatum* on Sabouraud Dextrose Agar

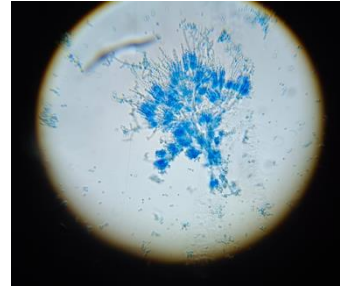


Fig-2: Microscopic observation of *Penicillium notatum*

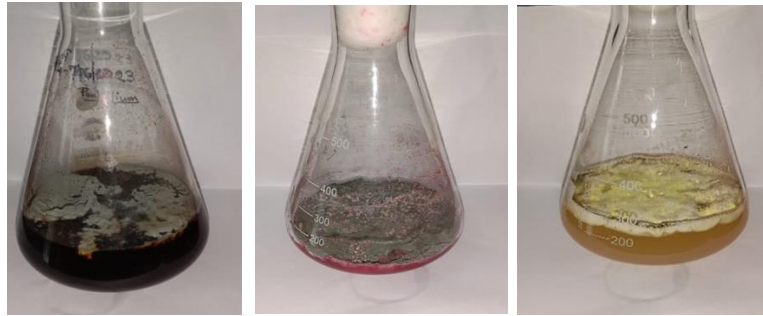


Fig-3: After 25 days of incubation, growth of *Penicillium sp.* in production media.



Fig-4: *Penicillium* mat



Fig-5: Crushing of *Penicillium* mat by mortar and pestle



Fig-6: Purification of broth



Fig-7: Extraction of mat

Plates showing Antimicrobial activity

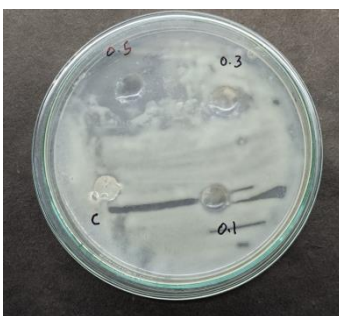


Fig-8: *Salmonella* sp.



Fig-9: *Vibrio* sp.



Fig-10: *Rodococcus* sp.



Fig-11: Klebsiella sp.

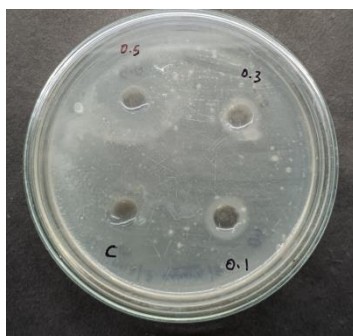


Fig-12: Staphylococcus aureus

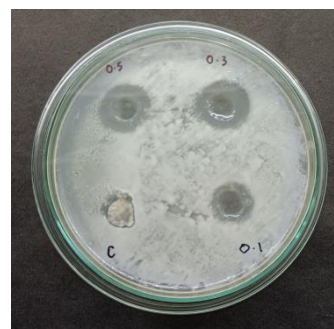


Fig-13: Staphylococcus cohnii

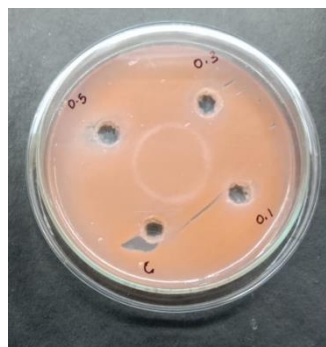


Fig-14: Pseudomonas sp.

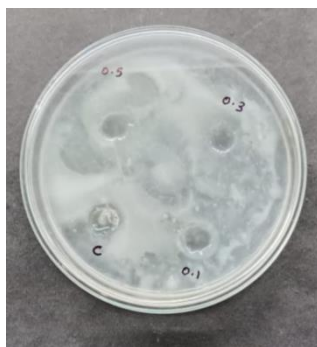


Fig-15: Bacillus sp.

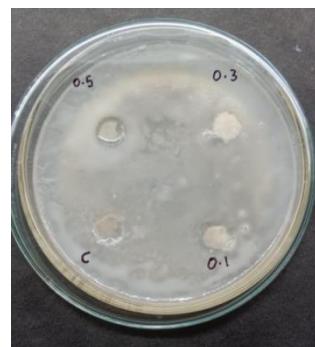


Fig-16: Candida sp.

Table-1: Antimicrobial activity of Penicillium against different microorganism

S. No.	Test Organisms	Zone of inhibition in diameter (mm)				Antimicrobial activity
		Control	0.1 ml	0.3 ml	0.5 ml	
1.	<i>Salmonella sp.</i>	-	1.25	1.35	2.5	Sensitive
2.	<i>Vibrio sp.</i>	-	1	2	2.25	Sensitive
3.	<i>Staphylococcus aureus</i>	-	1.45	1.35	2.85	Sensitive
4.	<i>Staphylococcus cohnii</i>	-	1.55	2	2.15	Sensitive
5.	<i>Pseudomonas sp.</i>	-	1.1	1.2	1.35	Sensitive
6.	<i>Rhodococcus sp.</i>	-	1	1.25	1.4	Sensitive
7.	<i>Klebsiella sp.</i>	-	1.35	1.45	1.6	Sensitive
8.	<i>Candida sp.</i>	-	-	-	-	Resistance
9.	<i>Bacillus sp.</i>	-	-	-	-	Resistance

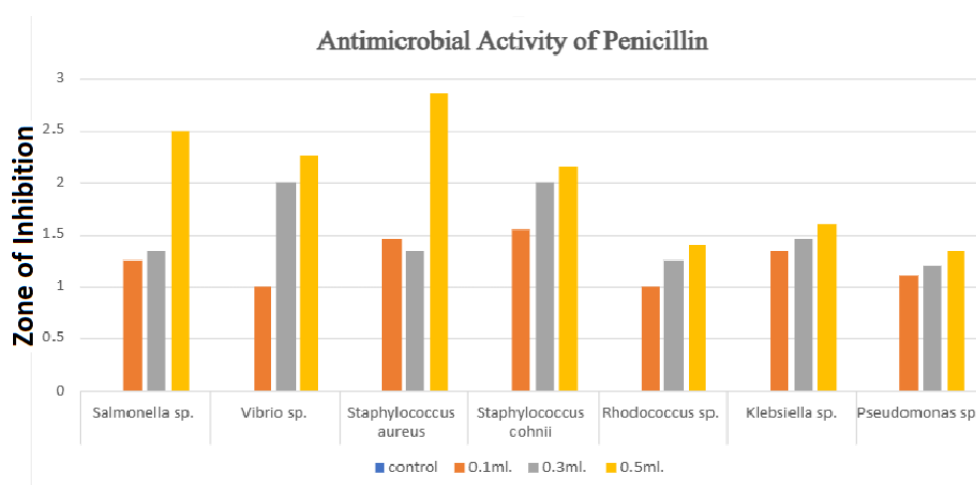


Fig-17: A graphical representation of in-vitro activity of Penicillin against various test organisms and form the zone of inhibition (mm).

DISCUSSION

Penicillium was isolated from the soil sample by performing serial dilution method and confirmed by lactophenol cotton blue staining observed under a 40X microscopic lens. Further using three different production media such as Potato Dextrose Broth (PDB), Rose Bengal Broth (RBB), Sabouraud Dextrose Broth (SDB) for penicillin production. After 25 days the yellow crystal penicillium mat was found only on the Sabouraud Dextrose Broth (SDB). From the extract of broth filtrate penicillin as a secondary metabolite was obtained and gave a positive result for the antimicrobial test by forming clear zones around the wells of plate inoculated with the test organisms (Dickinson *et. al.*, 1978).

This Data indicates the antagonistic property of Penicillin by antimicrobial analysis (Pisabarro *et. al.*, 1986). The strain used is *Penicillium notatum* produced using 3 different types of production media where wheat bran is used as a substrate. Penicillin, a secondary metabolite of particular strain, can be found in sabouraud dextrose broth through industrial means of production. To demonstrate penicillinase activity in vitro method against various microorganisms strains like *Salmonella sp.*, *Vibrio sp.*, *Staphylococcus aureus*, *Staphylococcus cohnii*, *Rhodococcus sp.*, *Klebsiella sp.*, *Pseudomonas sp.*, *Candida sp.*, *Bacillus sp.*. However, the penicillinase activity in the culture broth of *Penicillium notatum*, when minimal inhibitory concentration (MIC) procedures were tested in different concentrations (Khassaf *et. al.*, 2009).

The antimicrobial behaviour of penicillin, as compared with test organisms, can best be illustrated by an examination of the respective in-vitro means, as presented in Table 1. Concentrated preparations, from the purification of the broth were used. Some strains of *Bacillus* and *Candida species* show resistance to Penicillin but that most of the gram negative organisms isolates are sensitive by checking MIC against Penicillin (Niksic *et. al.*, 2018).

The majority of antibiotics that are now known to exist, such as penicillin and other mould compounds, work on bacteria. Numerous additional antibiotics have been developed as a result of the discovery of penicillin. One of the most significant and popular antibiotics in contemporary medicine is penicillin (Brook, 2016) Like penicillin, which has antagonistic qualities against a variety of organisms, these compounds' action towards organisms is quite selective. It is crucial to understand that penicillin only kills bacteria; it has no effect on human cells or some other types of microbes, such as viruses and fungi. Its safety and potency as an antibacterial therapy are aided by this specificity (Sabaa *et. al.*, 2015).

CONCLUSION

Penicillin is an excellent candidate to treat various infectious diseases but it is less effective against gram negative organisms, as well as bacterial resistance to the drug candidate, Where major development is required. The above research work concludes that high yield of penicillin production can be obtained using sabouraud dextrose broth medium with wheat bran as the substrate in industrial means. The filtrate obtained from broth after the incubation period possesses the antagonistic property which can inhibit the growth of bacterial test organisms.

DECLARATION

Ethical Approval and Consent to participate : Not Applicable

Consent for publication: I hereby give my consent for the use of my information, data, and likeness in publications, presentations, or reports related to the "COMPARATIVE ANALYSIS OF PENICILLIN PRODUCTION USING VARIOUS MEDIA COMPOSITIONS: A RESEARCH STUDY". I understand that my identity will remain confidential and my personally identifiable information will not be disclosed.

Availability of data and materials: The data and materials used in this thesis are available upon request from the author, subject to confidentiality and ethical considerations. For publicly available datasets, references and access instructions can be found in the bibliography.

Competing interests: The authors declare no competing interests .

Funding: Not Applicable

Authors' contributions: The contributions of the authors to this thesis are as follows: Sidra Khoucin and Sukanya Dutta, conceptualized the research design and conducted data collection, while Dr. S. Anu Kiruthika performed data analysis and interpretation. Both authors collaborated in writing and reviewing the manuscript.

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References

- Bodey GP, Stewart D. In vitro studies of semisynthetic alpha-(substituted-ureido) penicillins. *Appl Microbiol.* 1971 Apr;21(4):710-7. doi: 10.1128/am.21.4.710-717.1971. PMID: 4930281; PMCID: PMC377261.
- Bornside GH. Synergistic antibacterial activity of ampicillin-cloxacillin mixtures against *Proteus morgani*. *Appl Microbiol.* 1968 Oct;16(10):1507-11. doi: 10.1128/am.16.10.1507-1511.1968. PMID: 5188339; PMCID: PMC547693.
- Brook, I. (2016). Spectrum and treatment of anaerobic infections. *Journal of Infection and Chemotherapy*, 22(1), 1-13.
- Cycoń, M., Mroziak, A., & Piotrowska-Seget, Z. (2019). Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity. *Frontiers in microbiology*, 10, 338.
- Dede, A., Güven, K., & Şahin, N. (2020). Isolation, plant growth-promoting traits, antagonistic effects on clinical and plant pathogenic organisms and identification of actinomycetes from olive rhizosphere. *Microbial pathogenesis*, 143, 104134.
- Dickinson, G. M., Cleary, T. J., & Hoffman, T. A. (1978). Comparative evaluation of piperacillin in vitro. *Antimicrobial agents and chemotherapy*, 14(6), 919-921.
- Girolami, R. L., & Stamm, J. M. (1980). Comparative antimicrobial activity of O-demethyl fortimicin A, a derivative of fortimicin A. *Antimicrobial Agents and Chemotherapy*, 18(5), 766-772.
- Khassaf, H. K., & Almasoudi, W. A. (2009). Pharmacological study of Schiff base derived from amoxicillin drug and vanillin.
- Landesman, S. H., Cummings, M., Gruarin, A., & Bernheimer, H. (1981). Susceptibility of multiply antibiotic-resistant pneumococci to the new beta-lactam drugs and rosaramicin. *Antimicrobial Agents and Chemotherapy*, 19(4), 675-677.
- Lopez, H., Stepanik, D., Vilches, V., Scarano, S., Sarachian, B., Mikaelian, G., ... & Sucari, A. (2001). Comparative in vitro activity of gemifloxacin against gram-positive and gram-negative clinical isolates in Argentina. *Diagnostic microbiology and infectious disease*, 40(4), 187-192.
- Niksic, H. A., Duric, K., Omeragic, E., Niksic, H. E., Muratovic, S., & Becic, F. (2018). Chemical characterization, antimicrobial and antioxidant properties of *Mentha spicata* L.(Lamiaceae) essential oil. *Bulletin of the Chemists & Technologists of Bosnia & Herzegovina*, (50).
- Pisabarro, A. G., Prats, R. O. S. A., Vaquez, D., & Rodríguez-Tébar, A. L. F. R. E. D. O. (1986). Activity of penicillin-binding protein 3 from *Escherichia coli*. *Journal of bacteriology*, 168(1), 199-206.
- Rocha, A. J., Barsottini, M. R. D. O., Rocha, R. R., Laurindo, M. V., Moraes, F. L. L. D., & Rocha, S. L. D. (2019). *Pseudomonas aeruginosa*: virulence factors and antibiotic resistance genes. *Brazilian Archives of Biology and Technology*, 62.
- Sabaa, M. W., Abdallah, H. M., Mohamed, N. A., & Mohamed, R. R. (2015). Synthesis, characterization and application of biodegradable crosslinked carboxymethyl chitosan/poly (vinyl alcohol) clay nanocomposites. *Materials Science and Engineering: C*, 56, 363-373.
- Schatz, A., Bugle, E., & Waksman, S. A. (1944). Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria.*. *Proceedings of the Society for Experimental Biology and Medicine*, 55(1), 66-69.
- Schwartzkroin, P. A., & Prince, D. A. (1977). Penicillin-induced epileptiform activity in the hippocampal in vitro preparation. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 1(5), 463-469.
- Sharifzadeh, S., Brown, N. W., Shirley, J. D., Bruce, K. E., Winkler, M. E., & Carlson, E. E. (2020). Chemical tools for selective activity profiling of bacterial penicillin-binding proteins. In *Methods in enzymology* (Vol. 638, pp. 27-55). Academic Press.
- Vazquez-Muñoz, R., Meza-Villecas, A., Fournier, P. G. J., Soria-Castro, E., Juárez-Moreno, K., Gallego-Hernández, A. L., ... & Huerta-Saquero, A. (2019). Enhancement of antibiotics antimicrobial activity due to the silver nanoparticles impact on the cell membrane. *PLoS one*, 14(11), e0224904